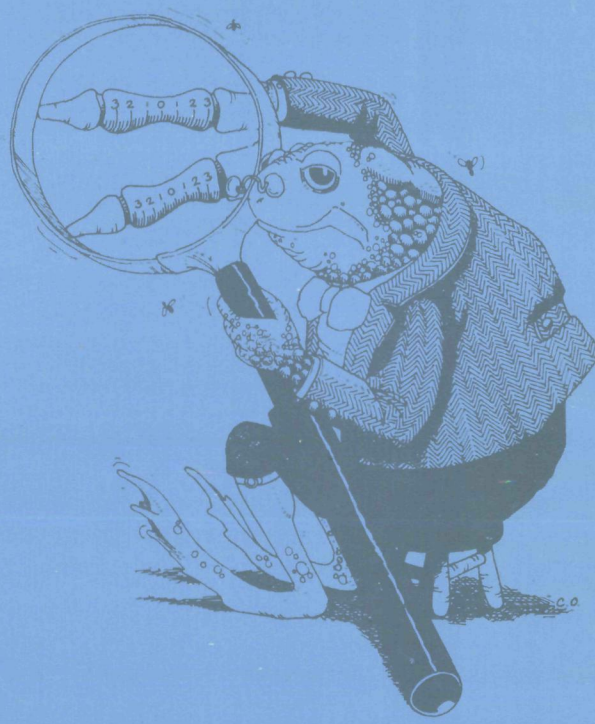
DEMOGRAPHIC STUDY ON *BUFO BUFO* L. (ANURA, AMPHIBIA) FROM DIFFERENT CLIMATES, BY MEANS OF SKELETOCHRONOLOGY



Agnes Hemelaar

DEMOGRAPHIC STUDY ON BUFO BUFO L. (ANURA, AMPHIBIA) FROM DIFFERENT CLIMATES, BY MEANS OF SKELETOCHRONOLOGY

Promotores: Prof. Dr. J.M. Denucé
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DEMOGRAPHIC STUDY ON BUFO BUFO L. (ANURA, AMPHIBIA) FROM DIFFERENT CLIMATES, BY MEANS OF SKELETOCHRONOLOGY

PROEFSCHRIFT
ter verkrijging van de graad van
doctor in de wiskunde en natuurwetenschappen
aan de Katholieke Universiteit te Nijmegen
op gezag van de Rector Magnificus
Prof. Dr. J.H.G I. Giesbers
volgens besluit van het College van Dekanen
in het openbaar te verdedigen
op donderdag 26 juni 1986
des namiddags te 4 00 uur

door
AGNES SIDONIA MARIA HEMELAAR
geboren te Rucphen

1986
Druk:Offsetdrukkerij Faculteit der Wiskunde en Natuurwetenschappen
Nijmegen

Aan allen die mij lief zijn

VOORWOORD

Graag wil ik op deze plaats iedereen bedanken die mij op een of andere wijze geholpen heeft bij het tot stand komen van dit proefschrift In de wetenschap niet volledig te zijn wil ik hier met name noemen

medewerkers en oud-medewerkers van de afdeling Dieroecologie met wie het prettig samenwerken was,

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Cees Omes die bewezen heeft een uitstekend en humoristisch illustrator te zijn,

familie en vrienden die, betrokken bij mijn doen en laten, mij morele steun gaven,

heel speciaal mijn ouders zij hebben zeer veel bijgedragen aan mijn vorming

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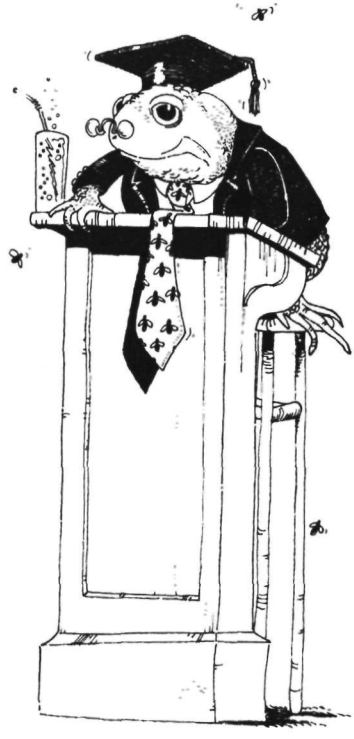


The importance of individual age determination in demographic studies on amphibians can hardly be over-estimated. Knowing the age of individuals in a population, it is possible to get information about longevity, mortality and growth rate at different ages and so on In former days, body sizes were usually used to classify individuals to age groups (Noble 1931, Tamsitt 1962 and, more recently, Bell 1977 and Daugherty and Sheldon 1982). However, the accuracy of this method is limited, since the large variability in body size among individuals of the same age mostly results in a broad overlap between the adjacent age groups (Senning 1940).

Accurate age determination of amphibians might be possible provided that their cyclic growth is recorded somewhere in the body structure of the animal. This has been shown true in fishes since long growth marks which can be used for age determination are present in scales, in otoliths and in particular bones (e.g. the opercular bone) (Menon 1950) In amphibians the cyclic growth is recorded in the skeleton. In analogy to dendrochronology, Castanet et al. (1977) introduced the term skeletochronology for it Growth marks were found on the surface and in unstained ground sections of flat bones (Senning 1940, Peabody 1961, Griffiths 1962, Schroeder and Baskett 1968). Kleinenberg and Smirina (1969) have shown that growth marks are also visible in hematoxylin-stained cross-sections of decalcified bone of *Rana temporaria,* a method introduced by Klevezal and Kleinenberg (1967) for mammalian bone. The best results were obtained from the long bones tibia and femur. Using the same method, Smirina (1972*a* 1972*b*) found also growth marks in the phalanges of *Bufo bufo* and *Rana temporaria.* Although Klevezal (1973) reported that growth marks in bones of mammals living in a semi-continental and oceanic climate are less distinct, Hemelaar (1980) demonstrated that they are clearly visible in the skeleton of *Bufo bufo* from a temperate oceanic climate. Three bones of the skull (pterygoid, parasphenoid and frontoparietal) and several phalanges were sectioned, stained and analysed. In all bones growth marks were present, but most distinct in the phalanges (Hemelaar 1980). This finding enables the analysis of growth marks in samples obtained by simple toe clipping, which does not necessitate the killing of the animal.

In phalanges the growth marks appear as concentric rings, the so-called growth rings (Kleinenberg and Smirina 1969). Growth rings are

relatively broad zones of periosteal bone, separated from each other by a narrow so-called "line of adhesion" (Klevezal and Kleinenberg 1967) or "resting line" (Kleinenberg and Smirina 1969). In hematoxylin-stained sections of decalcified bone the growth rings stain light, the resting lines appear dark (Klevezal and Kleinenberg 1967). More histological features of amphibian bone are described in detail by Castanet (1975 1979) and Castanet et al. (1977).

Smirina (1972*a* 1972*b*) showed that in phalanges of *Bufo bufo* and *Rana temporaria* from Russia only one growth ring and one resting line are formed each year. The growth ring is formed during the growing season and the resting line reflects the hibernation. As growth ring and resting line together refer to one year, they are referred to as a year ring. Year rings were also found by Schroeder and Baskett (1968) in *Rana catesbeiana* from Central Missouri. Both Smirina (1972*a* 1972*b*) and Schroeder and Baskett (1968) collected their material from an area with a (sub) continental climate.

In this thesis a method for individual age determination is worked out in detail, the application of which is not restricted to *Bufo bufo* from a particular climate. Furthermore, additional applications of skeletochronology in studies on the demography of common toads are presented.

In CHAPTER 1 of this thesis it is shown that year rings are formed in phalanges of adult *Bufo bufo* from a temperate oceanic climate. This is demonstrated using different phalanges of the same toads that were captured and recaptured during several years in nature. The age of those animals could be determined in principle by counting the number of year rings, present in the periosteal bone. In practice, however, this is not always possible. Bone, being a dynamic tissue, may be subjected to remodelling (Harris and Heany 1970). In long bones like phalanges, remodelling starts from the marrow cavity and involves the replacement of periosteal bone by endosteal bone. As a result initially formed year rings may be resorbed. The progress of the formation of endosteal bone is delineated by the resorption line, a narrow, irregularly formed, hematoxylinophilic line (Klevezal and Kleinenberg 1967).

The degree of resorption may vary in different taxa of amphibians. Urodela seem to have little or no resorption of year rings (Castanet 1975 and Castanet et al. 1977), whereas Smirina (1972*a* 1972*b*) found that in all adult *Bufo bufo* and *Rana temporaria* at the most remnants of the resting line of the second year ring were present. However, Hemelaar (1980 1981*a*) showed that resorption of year rings in bones of adult *Bufo bufo* from The

Netherlands may vary, both among individuals and among different bones of the same individual. In CHAPTER 2, a method is described to estimate individually the number of year rings resorbed in phalanges of adult male *Bufo bufo* from a temperate oceanic climate. The method is based on both the pattern of year rings in the phalanx of the adults and the phalangeal diameter in first-year toads towards hibernation. The degree of resorption of year rings appeared to be low. Only incidentally the first year ring has been resorbed in male toads from the populations studied.

However, this method might be less suitable when applied to female toads and to toads from populations in which the degree of resorption is higher. For that reason, the method was improved and applied to populations of *Bufo bufo* from different latitudes and altitudes. This is reported in CHAPTER 3. It is shown that the resorption of year rings may be considerable (up to 3 year rings) and that ignoring resorption can lead to as much as 76% error. With the improved method the number of year rings resorbed is estimated in 82-100% of the toads from the populations studied.

Thus, skeletochronology has proven to be an accurate method for individual age determination of *Bufo bufo*. Since the successive year rings in fact mark the annual phalangeal growth, it also offers a unique possibility to get information about the growth history of each individual toad, thereby adding a new dimension to demographic studies on amphibians. It may even be a valuable tool in studies on the taxonomic status of *Bufo bufo bufo* and *Bufo bufo spinosus* (Hemelaar 1984). In the last three chapters of this thesis, several demographic aspects of *Bufo bufo* are studied and discussed. Special attention is paid to age and age related characteristics, obtained by skeletochronology.

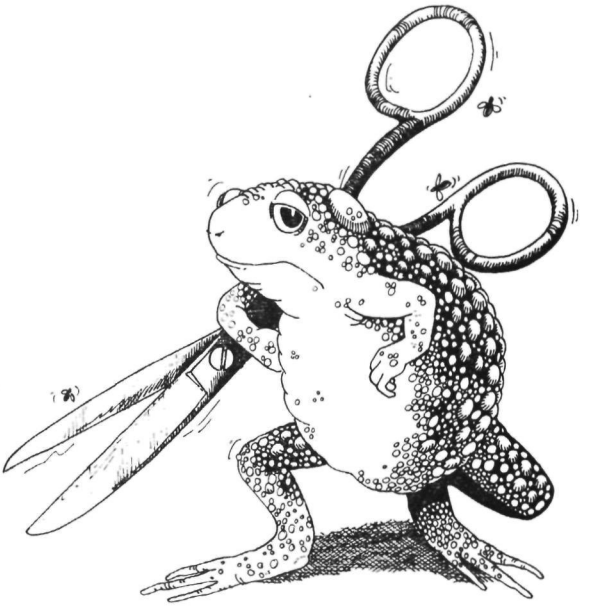
In CHAPTER 4 pairs of toads from a spawning population living in a temperate oceanic climate are studied. The age structure of both sexes indicates that female toads generally mature at least one year later than male toads and may reach an older age. It is concluded that the observed imbalance in the sex ratio of the spawning population -males outnumber females- is mainly due to the delayed maturation of the female toads.

In CHAPTER 5 the age structure of the same spawning population in two successive years is discussed. It is demonstrated that the younger age classes migrate only partially to the spawning site, as a consequence of the fact that the age of maturation is variable. Furthermore, the growth history of toads is described for each cohort (= group of toads born in the same year). The results suggest that the differences in growth at comparable

ages, found among cohorts, are caused by variations in the environmental conditions during growth.

Finally, in CHAPTER 6 populations from different climatological conditions are compared. The age structures of the spawning populations are presented and the annual growth of toads of different ages is analysed. Intra- and interpopulational differences and similarities with respect to age structure, size, longevity, age and size at maturation, annual growth and several other parameters are discussed. When the actual time spent on growth each year was considered, growth appears to proceed equally among most populations. The sexual dimorphic growth coefficient found is assumed to represent a constant which describes all linear growth processes that are sexually dimorphic. The minimum time to be spent on growth before reaching the minimum size of maturation, calculated with the growth equations, also appears to be constant among most populations. With this constant it is possible to estimate the average minimum age of maturation in populations of common toads without knowing the age of the individuals.

YEAR RINGS IN PHALANGES OF BUFO BUFO FROM A TEMPERATE OCEANIC CLIMATE.



Modified version of:

Hemelaar, A.S.M. and J.J. van Gelder (1980). Annual growth rings in phalanges of Bufo bufo (Anura, Amphibia) from the Netherlands and their use for age determination. Neth. J. Zool. 30: 129-135.

INTRODUCTION

A promising method for individual age determination of amphibians seems to be the analysis of growth marks in their skeleton (Senning 1940, Peabody 1961, Schroeder and Baskett 1968, Kleinenberg and Smirina 1969). In phalanges, the growth marks appear as relatively broad layers of periosteal bone -the growth rings- separated from each other by a narrow resting line (Kleinenberg and Smirina 1969, Smirina 1972*a* 1972*b*). Smirina (1972*a* 1972*b*) found that in phalanges of *Bufo bufo* and *Rana temporaria* from Russia only one growth ring and one resting line are formed each year. Together they form a year ring. Such year rings are also found in *Rana catesbeiana* (Schroeder and Baskett 1968) and most likely also in *Triturus alpestris* (Smirina and Roček 1976).

Age determination by counting the number of year rings in the periosteal bone of phalanges may be hindered, however, by several factors. First of all, bone may be subjected to remodelling. With respect to phalanges, this means that periosteal bone is replaced by endosteal bone, starting from the marrow cavity As a result, initially formed year rings may be resorbed. The progress of the endosteal bone is marked by the resorption line (Klevezal and Kleinenberg 1967). Second, the identification of the year rings may sometimes be hampered by the occurrence of "supplementary streaks" and "double lines" (Klevezal and Kleinenberg 1967), although Kleinenberg and Smirina (1969) and Smirina (1972*a* 1972*b*) mentioned that these additional structures did not cause serious problems in age determination. Finally, Klevezal (1973) showed that the year rings in bones of mammals from a semi-continental and oceanic climate are generally less distinct than in those from a continental climate According to Klevezal (1973) caution should be exercised when year rings are used for age determination in animals from more temperate climates

The question whether in phalanges of *Bufo bufo* from a temperate oceanic climate year rings are formed which can be used for age determination, is being dealt with in this chapter.

MATERIAL AND METHODS

From 1975 to 1978, adult common toads, *Bufo bufo*, were captured at 2 spawning sites, called Feuerloschteich and Drie Meertjes, situated in the Reichswald and Sint Jansberg respectively, both about 13 km south-east of

Nijmegen. Snout-urostyle length was measured to the nearest mm and after marking by toe clipping the toads were released. Toes were separately stored at -20° C, awaiting further treatment. From the material collected over the 4 years of sampling, toes were selected that had been removed from the same toads, but in different years (see Fig. 1 1). Altogether, a total of 89 toads was available for the present study.

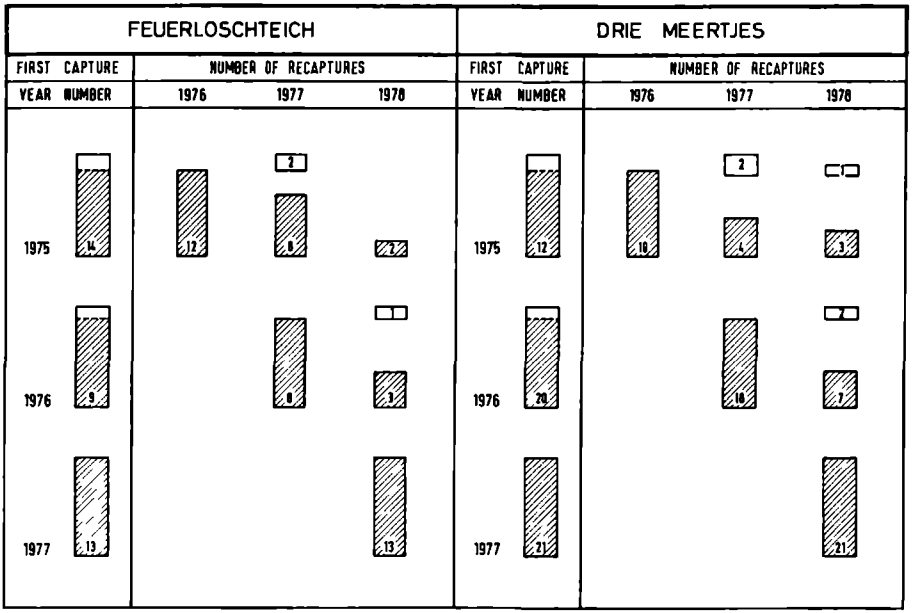


Fig 1.1 *Survey of capture-recapture data of toads selected for this study. Horizontal equally shaded columns refer to the same toads.*

From the toes selected only the last but one phalanx was used. After boiling in tap water for 30 minutes, the phalanges were cleaned and decalcified in 5% formic acid for at least 1 hour The decalcifying agent was removed by rinsing the phalanges in distilled water for about 20 hours. Samples that were not to be sectioned immediately were fixed in 70% ethanol. Before sectioning the phalanges were hydrated (if fixed) and frozen in water at -20° C. Cross-sections of 20 µm, made with a freezing microtome (Jung, Heidelberg), were attached to slides with albumen glycerin (Chroma-Gesellschaft, Stuttgart) The sections were stained with Delafield's hematoxylin (Romeis 1948) for about 30 minutes. Finally, they were rinsed

in tap water (15 minutes), mounted in Kaiser's glycerol gelatin (Merck, Darmstadt) and protected with glass cover-slips.

The sections were examined with a Zeiss light microscope. The number of growth rings was determined by counting the number of resting lines, including resting lines affected by resorption. According to Smirina (1972*b*), who stated that in adult *Rana temporaria* bone growth starts only after the reproduction period, the outer margin of the bone was also considered a resting line. Only sections through the middle of the diaphysis were used, as they contain the largest amount of periosteal bone, allowing the resting lines to be distinguished here the best.

RESULTS

In all cross-sections examined resting lines could be clearly distinguished. Plate 1 1a shows a cross-section with 5 complete resting lines and 1 that has been partly resorbed by the progress of the endosteal bone. In the outer margin the resting line that should mark the preceding growth ring is only slightly visible. Nevertheless, 7 growth rings can be distinguished, showing the normal pattern of decreasing bone growth with increasing age. In some sections this pattern was disturbed by the presence of 2 resting lines which were very close to each other, often showing fusion at some particular point (Plate 1 1e, d.l.). These 2 resting lines were considered 1 resting line that is doubled. Consequently, they added only 1 to the total number of growth rings. Besides this, some sections showed structures which resembled resting lines. As they were rather vague and mostly incomplete, these structures were not considered resting lines. They will be called false lines (Plate 1.1d).

Table 1.1. *Increase in the number of growth rings in recaptured toads. n = number of toads, n_i = number of toads recaptured after i years, r_i = number of toads in sample n_i with an increase of i growth rings.*

collecting site	n	n_1	r_1	n_2	r_2	n_3	r_3	$(\Sigma r_i/\Sigma n_i)\times 100$
Feuerloschteich	36	33	32	14	11	2	2	91.5
Drie Meertjes	53	49	47	15	13	4	4	94.1
total	89	82	79	29	24	6	6	93.2

Plate 1.1.

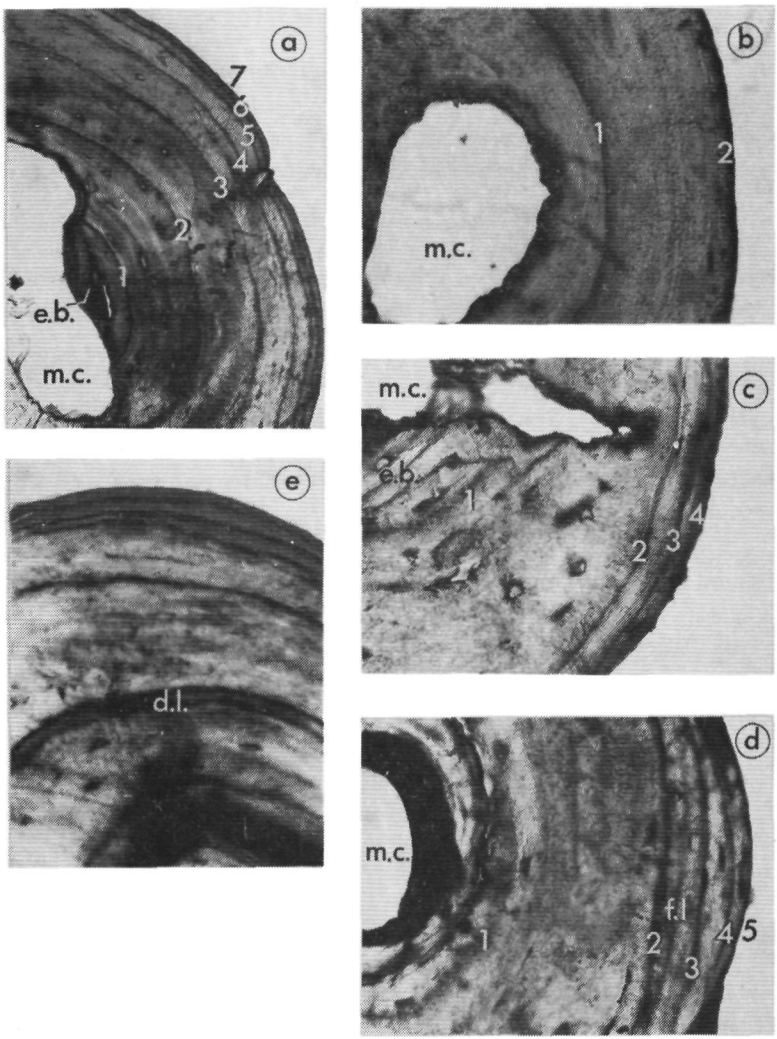


Plate 1.1. *Hematoxylin-stained cross-sections of phalanges of Bufo bufo. Resting lines are numbered. m.c. = marrow cavity. e.b. = endosteal bone. a. Animal with 7 growth rings (see text). b-d. Animal captured in 1975 (b) and recaptured in 1977 (c) and 1978 (d), f.l. = false line. e. Cross-section showing a double line (d.l.).*

The results of the analyses are given in Table 1.1. In more than 90% of the toads recaptured from both collecting stations, the increase of the number of growth rings was equal to the difference in years between capture and recapture, indicating an average increase of 1 growth ring a year. From the fact that many toads were captured in 3 (and some in 4) successive years (Fig. 1.1) and that the toads in which the number of growth rings deviated from the expected number did not come from one particular year (Table 1.2), it may be concluded that indeed only 1 growth ring a year is formed in adult *Bufo bufo* from the populations studied. Plate 1.1b-1d illustrates this for an animal captured in 3 years.

Table 1.2. *Number of toads captured (n_c) in the two years concerned and number of toads having formed one growth during that period (n).*

collecting site	1975-1976		1976-1977		1977-1978	
	n_c	n	n_c	n	n_c	n
Feuerlöschteich	12	12	16	15	18	16
Drie Meertjes	10	10	22	20	32	31

The number of growth rings in toads at their first capture varied from 2 to 7 (Fig. 1.2), in the recaptures up to 10 growth rings were counted.

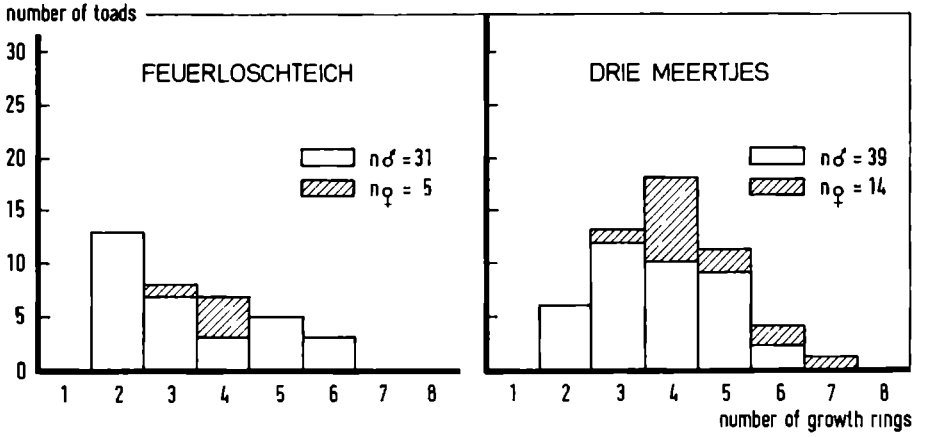


Fig. 1.2. *Number of growth rings in toads at their first capture.*

The method used is very similar to the one which Kleinenberg and Smirina (1969) applied to *Rana temporaria*. Some modifications were introduced, such as reduction of the time of decalcification to 60-90 minutes, and staining with Delafield's hematoxylin.

The false lines, occurring in some sections, did not cause serious problems in determining the number of growth rings. In addition to their structural identification, one can easily recognize them when they lie within initially formed growth rings and disturb the normal pattern of growth rings. Undoubtedly, these false lines correspond to the "supplementary streaks" described by Klevezal and Kleinenberg (1967). These authors assumed that a false line might be formed during an unfavourable period in summer. Nevertheless, the possibility cannot be excluded that the false line marks indeed the growth ring of a year in which bone growth is disturbed for some reason.

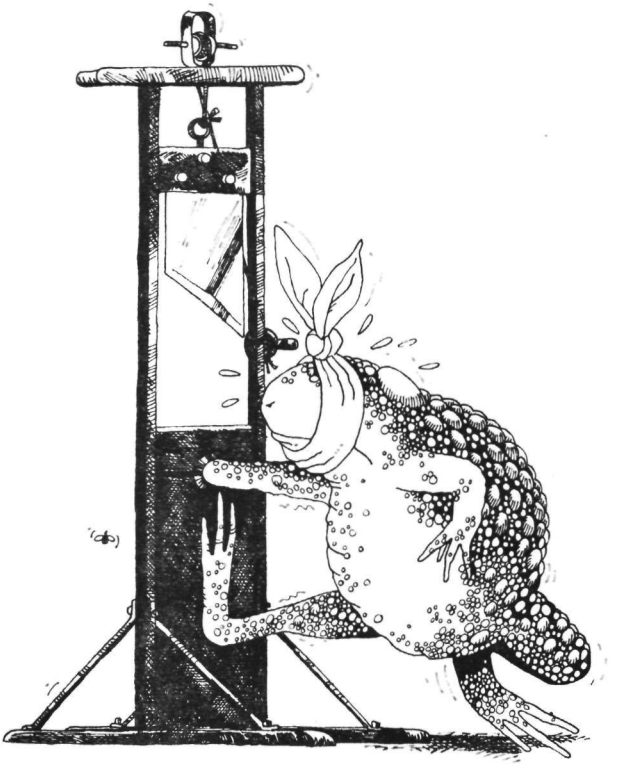
Besides these false lines, double lines also occurred occasionally. Klevezal and Kleinenberg (1967), who also described these double lines, suggested that they may be caused by a short interruption of hibernation (e g during a mild period in winter). When a double line is formed during the years of rapid bone growth, one can easily recognize it, as it disturbs the normal pattern of growth rings. At a more advanced age growth rings are small, thus making identification of a double line more difficult. However, it is believed that a short interruption of hibernation at an older age would hardly cause any bone growth, and, hence, would not result in the formation of a double line either.

Less than 10% of the sections did not show the expected number of growth rings Apart from possible errors, caused by the false lines mentioned above, it is possible that mistakes were made with identification of the toads Indeed, toads may lose toes in nature which may result in false recapture data. Differences between the expected and the observed number of growth rings might also be caused by resorption of one or more growth rings between capture and recapture. However, judging from the pattern of growth rings, it was possible to decide for each individual toad whether a growth ring had been resorbed between capture and recapture. Thus, resorption is not considered to be a factor responsible for the 10% mentioned above.

Since in 3 successive years more than 90% of the toads have formed 1 growth ring each year, it is concluded that in adult *Bufo bufo* from a

temperate oceanic climate year rings are formed. But, individual age determination is still hampered by potential resorption of initially formed growth rings. Smirina (1972*a*) concluded that in adult *Bufo bufo* always 2 year rings are affected by resorption. On the basis of the results, presented in this chapter, her conclusions cannot be confirmed for adult toads from a temperate oceanic climate. In some sections complete resting lines were seen and such a small marrow cavity, that it is hard to believe that in this particular phalanx 2 year rings (or even 1) have been resorbed A method has to be developed to determine for each individual toad the number of year rings resorbed. Only then individual age determination of *Bufo bufo,* using year rings in phalanges, will be possible, even in areas with a temperate oceanic climate.

AGE DETERMINATION OF MALE BUFO BUFO FROM A TEMPERATE OCEANIC CLIMATE, BASED ON YEAR RINGS IN PHALANGES.



Modified version of:

Hemelaar, A.S.M. (1981b). Age determination of male Bufo bufo (Amphibia, Anura) from the Netherlands, based on year rings in phalanges. Amphibia-Reptilia 1: 223-233.

INTRODUCTION

It is now generally accepted that the annual rhythm of growth in amphibians is recorded in their skeleton, resulting in the formation of year rings. (Senning 1940, Peabody 1961, Schroeder and Baskett 1968, Kleinenberg and Smirina 1969, Smirina 1972*a* 1972*b*, Castanet 1975, Smirina and Roček 1976, Hagstrom 1977, Francillon 1979, Barbault et al. 1979). Formation of year rings in bones of amphibians is not restricted to animals from the (sub)continental climate of the cool-temperate zone (Schroeder and Baskett 1968, Smirina 1972*a* 1972*b*), as is shown in chapter 1 for *Bufo bufo* from a temperate oceanic climate and by the study of Barbault et al. (1979) on the desert species *Bufo pentoni* from Senegal (tropical zone), where humidity controls the annual rhythm of bone growth. However, age determination based upon counting the number of year rings in phalanges of the animal is still hindered by the fact that initially formed year rings may be resorbed, as a result of remodelling of the bone. Resorption of year rings is believed to decrease sharply after reaching maturity (Smirina 1972*a* 1972*b*) By that time, the year ring corresponding to the first year of life has been resorbed completely, while, at the most, only remnants of the resting line of the second year ring may be present (Smirina 1972*a* 1972*b*). This phenomenon was also observed by Barbault et al (1979) in *Bufo pentoni*. Consequently, Smirina (1972*a* 1972*b*) determined the age of *Bufo bufo* and *Rana temporaria* by counting the number of intact year rings and adding the 2 resorbed ones to this value. However, from chapter 1 it appears that there was reason to suppose that this does not apply to adult *Bufo bufo* from a temperate oceanic climate.

This chapter presents a method to estimate individually the number of year rings resorbed in phalanges of *Bufo bufo* from a temperate oceanic climate for the purpose of individual age determination

MATERIAL AND METHODS

Within the scope of a study on the population dynamics of *Bufo bufo*, adult toads were captured from 1975 to 1979 at two spawning sites, Feuerlöschteich and Drie Meertjes (see chapter 1), situated about 1½ kilometres from each other. Toes which were clipped for marking were separately stored at -20° C. As the present study required the use of the same phalanx of toads of the same sex, male toads were selected from the

samples, of which only the third toe of the left forefoot (ft) was removed. Thus, ft of 86 males from Feuerlöschteich and of 121 males from Drie Meertjes became available. These males were mainly recaptures from previous years. Only the last but one phalanx of this particular toe was used for this study.

After a 5 hour treatment with 1-2% trypsin at 60° C, the phalanges were treated according to the method described in chapter 1. Only the mounting agent was replaced. The sections were mounted in Aquamount (Gurr).

The sections were analysed as described in chapter 1. Starting from the marrow cavity, the diameter of the first 3 year rings (indicated as $\emptyset_1$, $\emptyset_2$ and $\emptyset_3$, respectively) were measured (µm). Each diameter was measured on both the longest and the shortest axes which were perpendicular to each other. The two measurements were multiplied and the square root was extracted of this product. Measurements were made in four diaphyseal sections of each phalanx. These sections were defined as those of which the outer diameter was the smallest. The average value was calculated from the four square roots, thus providing the average phalangeal diameter at the end of each growth period. The diameter of the endosteal bone ($\emptyset_{rl}$), delineated by the resorption line, was measured in the same way.

Additionally, in the summer and autumn of 1979 newly metamorphosed toads were captured around Feuerlöschteich and Drie Meertjes. The ft of these toads was removed and treated with 1-2% trypsin for about one hour and cleaned. The diaphyseal diameter of this phalanx was then measured without sectioning.

RESULTS

In nearly all sections examined the resting lines were sufficiently distinct to count the year rings and to measure their diameters (Plate 2.1a). In some sections, however, the diameter of the first year ring could not be measured, because only remnants of it were present (Plate 2.1b). This applies to 14 males from Drie Meertjes and to 5 males from Feuerlöschteich. Occasionally, only 2 year rings were present and sometimes the third year ring was not sufficiently distinct to measure its diameter. Consequently, the number of toads in which the diameter of a particular year ring was measured varies.

In fact, by measuring the diameter of a particular year ring, the

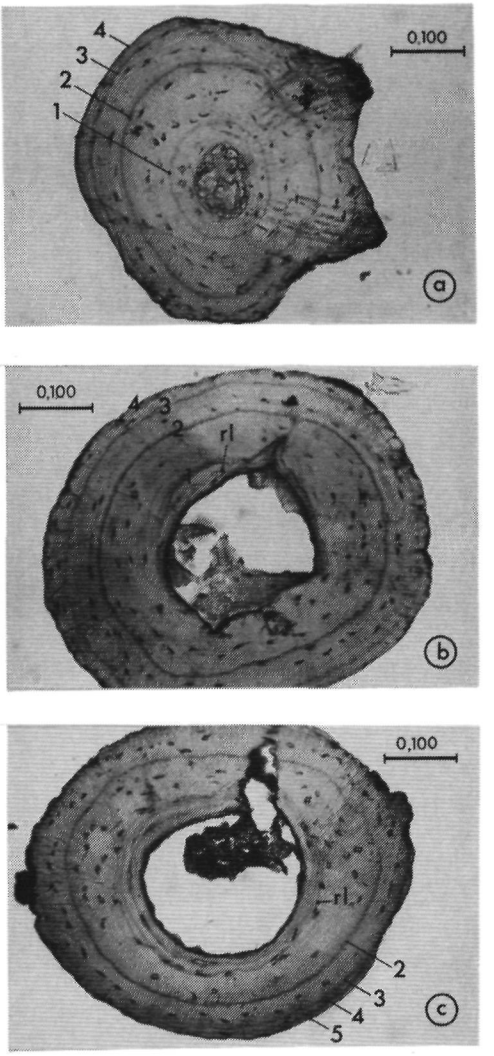


Plate 2.1. *Hematoxylin-stained cross-sections of phalanx ft of male toads. Resting lines are numbered. rl = resorption line.*
a. Toad in which no year ring has been resorbed. b. Toad in which the first year ring has been resorbed partially. c. Toad in which the first year ring has been resorbed completely.

phalangeal diameter at a particular age is measured. Based on the phalangeal diameter of 2 successive year rings the relative phalangeal growth y_i was assessed according to $(\emptyset_{i+1}-\emptyset_i)/\emptyset_i$. Table 2.1A shows that both the phalangeal diameter and the relative phalangeal growth differ highly significantly in two successive years. From this Table it also appears that the toads from the two study areas differ markedly. In Fig. 2.1 and Fig. 2.2 the phalangeal diameter is plotted versus the relative phalangeal growth for toads from Drie Meertjes and Feuerlöschteich, respectively. Based on the large $\emptyset_1$ as well as the low relative phalangeal growth y_1 in 7 males from Drie Meertjes and in 2 males from Feuerlöschteich (indicated with arrows), it is concluded that in these toads $\emptyset_1$ measured is actually $\emptyset_2$, and, accordingly, $\emptyset_2$ measured is $\emptyset_3$. In other words, at least one year ring has been completely resorbed in these toads (Plate 2.1c). As a consequence of this resorption Table 2.1A has to be revised, resulting in Table 2.1B.

Table 2.1. *Diameter of year rings and relative phalangeal growths in male toads. $\emptyset_i$ = diameter of year ring i, y_i = relative phalangeal growth = $(\emptyset_{i+1}-\emptyset_i)/\emptyset_i$, n = number of toads, av = average, sd = standard deviation, T = statistic, 3M = Drie Meertjes, FLT = Feuerlöschteich.*

	$\emptyset_1$			$\emptyset_2$			T	y_1			y_2			T
	n	av	sd	n	av	sd		n	av	sd	n	av	sd	
A. Without correction for resorption														
3M	107	201	46	121	332	43	a	107	.713	.239	114	.143	.068	a
FLT	81	209	35	86	374	34	a	81	815	.218	78	.121	.072	a
T		b			b				c			c		
B. With correction for resorption														
3M	100	191	28	121	328	40	a	100	.750	.206	116	.152	.070	a
FLT	79	205	26	86	372	32	a	79	.830	.199	80	.123	.074	a
T		c			b				c			c		

a = Student's t-test for matched pairs, b = Mann-Whitney U-test, c = Student's t-test, all $p<.01$.

The relative phalangeal growth decreases exponentially with increasing phalangeal diameter within each age class, as can be seen in Fig. 2.1 and Fig. 2.2. Each regression curve (corrected for the resorption mentioned and outliers excluded) is highly significant ($P<0.01$).

Theoretically, it is possible that in all toads at least the first year ring

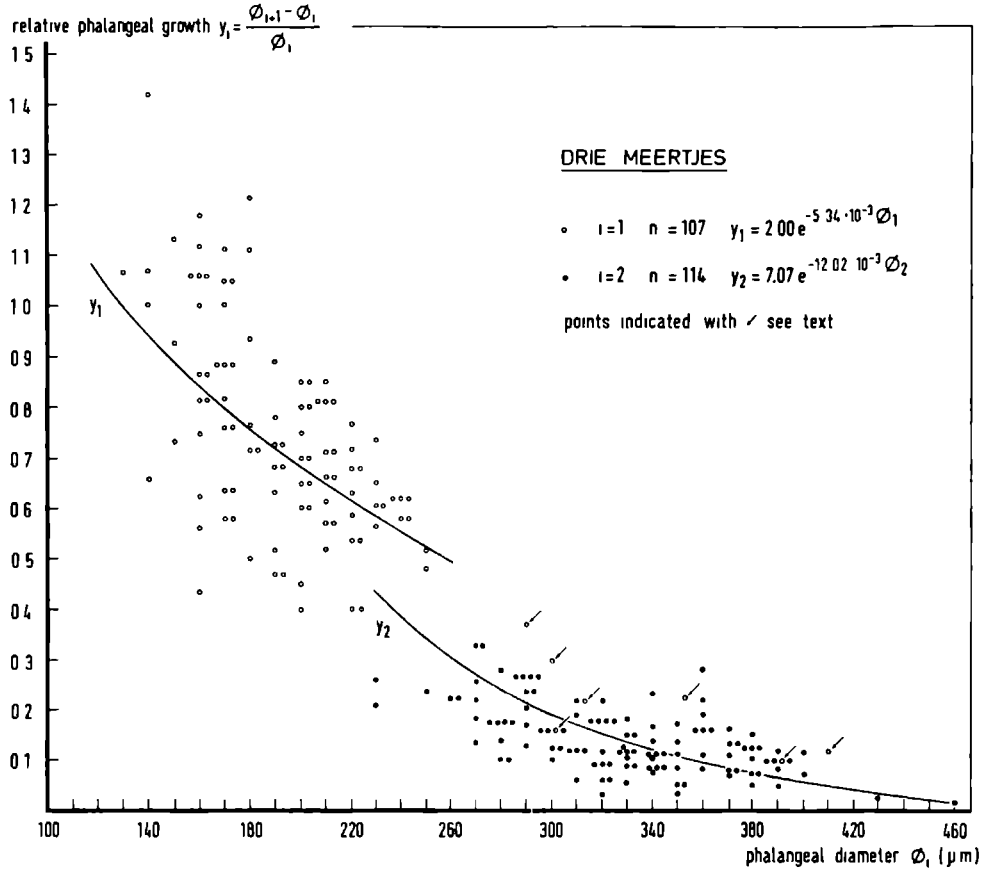


Fig. 2.1. *Relation between the diameter of year ring i and the relative phalangeal growth y_i in male toads from Drie Meertjes.*

has been resorbed completely, so that $\emptyset_1$ measured reflects at least $\emptyset_2$. In order to get an idea of the phalangeal diameter at the end of the first year, young newly metamorphosed toads were captured around the ponds from metamorphosis towards hibernation The phalangeal diameter in these toads is given in Figure 2.3. As it was assumed that both sexes have equal phalangeal diameters in their first year (see chapter 3), the young toads were not divided according to sex. Metamorphosis of tadpoles from Drie Meertjes took about 2 months, from July 8 until September 10. The dispersal of the young toads was followed until they mingled with those from Feuerlöschteich.

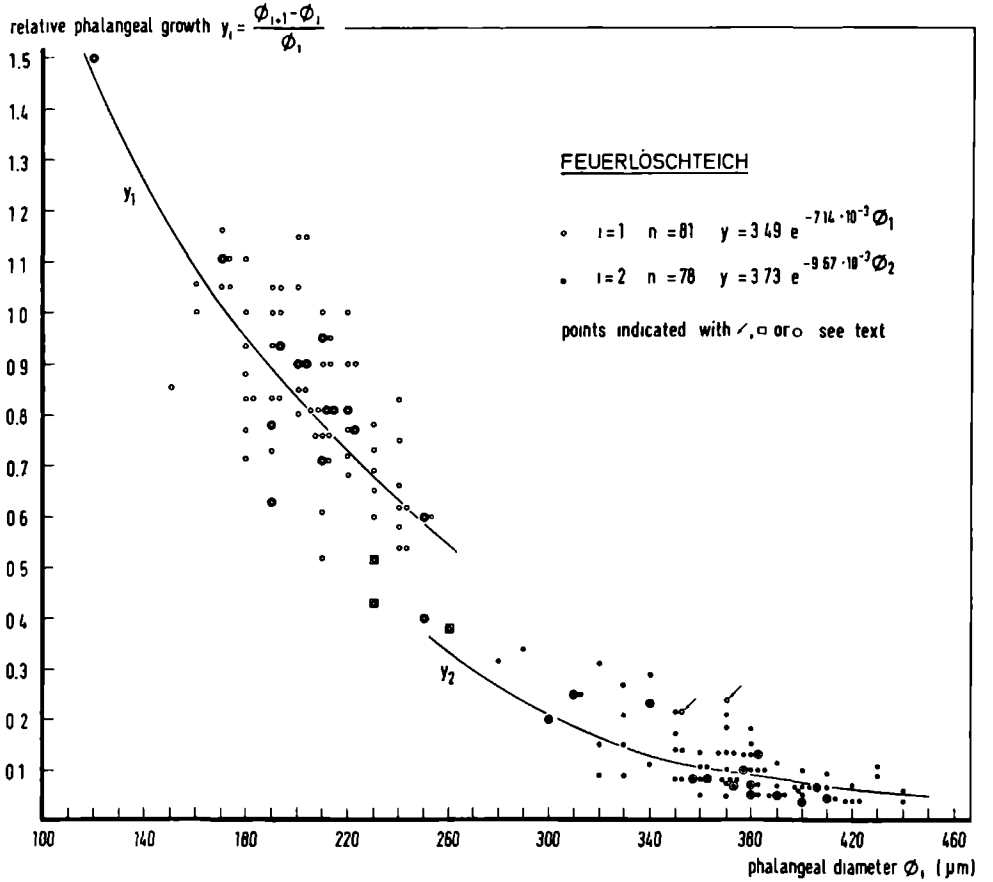


Fig. 2.2. *Relation between the diameter of year ring i and the relative phalangeal growth y_i in male toads from Feuerlöschteich.*

The phalangeal diameter has then increased from 100 ± 9 µm in July to 161 ± 19 µm (ranging from 121 to 198) in September, one week after the last tadpoles from Drie Meertjes had metamorphosed. In the adult males from Drie Meertjes $\emptyset_1$ measured ranges from 130 to 250 µm, averaging 191 µm (see Table 2.1B). Since the young toads still have time for phalangeal growth before hibernation, it is concluded that $\emptyset_1$ measured in the adults indeed corresponds to the diameter of the first year ring. The first year ring has been resorbed in only 7 out of the 121 adult toads.

In Feuerlöschteich metamorphosing tadpoles were present about 3 months (see Fig. 2.3). In collecting the young toads around the pond their origin

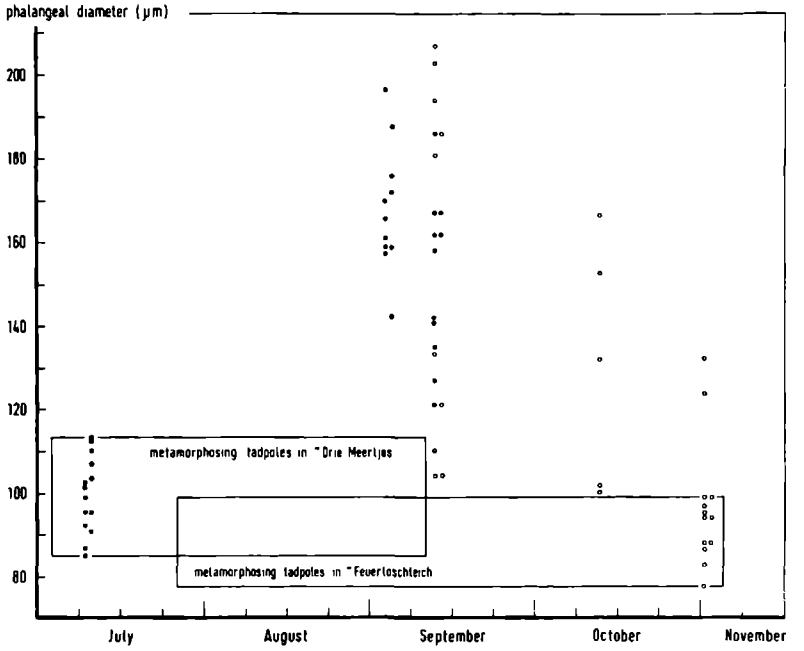


Fig. 2.3. *Period of metamorphosis of tadpoles and phalangeal diameter in first-year toads from metamorphosis onwards.*
Drie Meertjes = •, *Feuerlöschteich* = o.

could not be established with certainty, as part of the young toads from Drie Meertjes had reached Feuerlöschteich already. Thus, the phalangeal diameter at the end of the first year in toads from Feuerlöschteich cannot be 'predicted'. Anyway, the minimum phalangeal diameter at that time will be 90 µm, as being measured in the latest metamorphosed toads (early November). As Fig. 2.2 shows, all $Ø_1$ measured in the adult males from Feuerlöschteich are considerably larger, except for one small $Ø_1$ of 120 µm. Thus, the possibility remains that in a number of these males the first (small) year ring has been resorbed, $Ø_1$ measured actually being $Ø_2$. Since in 15 toads (Fig. 2.2, encircled) no endosteal bone was present, it is sure that in these animals the first year ring has not been resorbed. $Ø_1$ and $Ø_2$ in these toads do not differ from those in the other toads (Mann-Whitney *U*-test, both $P>0.24$), neither do their relative phalangeal growths (Mann-Whitney *U*-test, both $P>0.20$). Therefore, the exponential equation for estimating the relative phalangeal growth from Fig. 2.2 may be used to

estimate the 95% confidence limits of y_1 in late metamorphosed toads with small $\emptyset_1$ (Table 2.2). Knowing these limits, those of $\emptyset_2$ can be estimated. Again, the relative phalangeal growth in the next year can be estimated from the exponential equation and the estimated $\emptyset_2$. Consequently, if in adult toads from Feuerlöschteich the $\emptyset_1$ measured is between the 95% confidence limits of $\emptyset_2$, given in Table 2.2, and the y_1 measured is not larger than the corresponding y_{2max}, it is possible that in these toads the first small (90-130 µm) year ring has been resorbed. Besides the 2 males, already being identified as having resorbed at least 1 year ring (indicated with arrows), Fig. 2.2 shows only 3 more males (indicated with squares) that might be considered as having resorbed the first year ring. From these data it is concluded that $\emptyset_1$ measured in adult males from Feuerlöschteich generally represents the diameter of the first year ring. Only in 2 and maybe in 3 other males out of 86, the first year ring has been resorbed.

Table 2.2. *The 95% confidence limits of y_1 and $\emptyset_2$ and the maximal y_2 (y_{2max}) in late metamorphosed toads from Feuerlöschteich with small $\emptyset_1$. For statistical procedure see Mendenhall et al. (1977). Further explanation see text. $\emptyset_{2min}$ = minimum $\emptyset_2$.*

$\emptyset_1$	95% confidence limits		$\emptyset_{2min}$	y_{2max}
	y_1	$\emptyset_2$		
90	1.402-2.268	216-294	216	0.629
100	1.281-2.137	228-314	228	0.575
110	1.169-2.011	239-331	239	0.532
120	1.064-1.900	248-348	248	0.499
130	0.967-1.791	256-363	256	0.474

To find out if more than one year ring has been resorbed in those toads in which the first year ring was (or might be) resorbed, the diameter of the endosteal bone, delineated by the resorption line ($\emptyset_{rl}$), has to be considered. $\emptyset_{rl}$ in these toads (indicated with arrows or squares in Fig. 2.1 and Fig. 2.2) from Drie Meertjes and Feuerlöschteich ranged from 180 to 250 µm and from 130 to 290 µm, respectively. According to Table 2.1B it should be kept in mind that, when estimating the number of year rings resorbed:

- within $\emptyset_{rl}$, year rings should be reconstructed in such a way that their

diameters agree with those at that particular age,
- the reconstructed year rings should be arranged in such a way that the relative phalangeal growths agree with those at that particular age.

In only 1 male from each site more than 1 year ring can be reconstructed satisfying both conditions.

Table 2.3. *Degree of resorption of year rings in adult male toads. n = number of toads. 3m = Drie Meertjes. FLT = Feuerlöschteich.*

	3M		FLT		total	
	n	%	n	%	n	%
$\emptyset_1$ not resorbed	100	82.6	76	88.4	176	85.0
$\emptyset_1$ partially resorbed	14	11.6	5	5.8	19	9.2
$\emptyset_1$ maybe resorbed			3	3.5	3	1.4
$\emptyset_1$ resorbed	6	5.0	1	1.2	7	3.4
$\emptyset_1$ and maybe $\emptyset_2$ resorbed	1	0.8	1	1.2	2	1.0
total	121	100.0	86	100.0	207	100.0

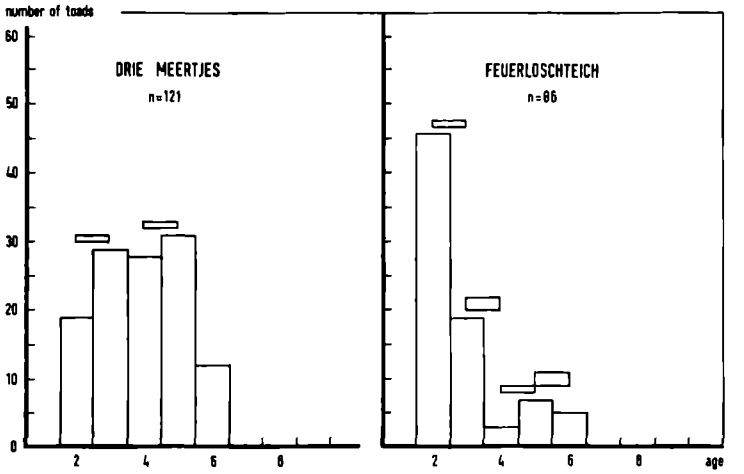


Fig. 2.4. *Age of male toads at their first capture. Blocks placed in between 2 years refer to toads the age of which could not be assigned to any of these years with certainty.*

A survey of the results concerning the degree of resorption of year rings in adult males is given in Table 2.3. In more than 94% of the males from both sites the resting line of the first year ring or remnants of it, could be observed. Only in 6% of the males from Drie Meertjes and in 2½ to 6% of those from Feuerlöschteich the first year ring has been resorbed completely. From these toads there is only 1 from each site that might be considered having resorbed 2 year rings.

The age of the toads at their first capture is represented in Fig.2.4 for both sites. Besides the toads in which the degree of resorption of year rings was unknown, the age of 1 male from Drie Meertjes and that of 2 from Feuerlöschteich could not be estimated exactly, as the number of year rings in their phalanges was dubious. The age of these toads might be underestimated by not more than 1 year.

DISCUSSION

As already mentioned in chapter 1, the method used is most useful to determine the number of year rings in phalanges of *Bufo bufo* from a temperate oceanic climate. The few males , 2 from Feuerlöschteich (2%) and 1 from Drie Meertjes (1%) in which the resting lines were not sufficiently distinct to count the number of year rings do not invalidate this conclusion.

Based on the pattern of year rings (expressed as the relation between phalangeal diameter and relative phalangeal growth) and the phalangeal diameter in newly metamorphosed toads just before hibernation, it was possible to identify those toads in which at least 1 year ring had been resorbed. The low degree of resorption found in this study is in sharp contrast with the results reported by Kleinenberg and Smirina (1969), Smirina (1972*a*) and Barbault et al. (1979) for *Rana temporaria, Bufo bufo* and *Bufo pentoni,* respectively. Kleinenberg and Smirina (1969) and Smirina (1972*a*) assigned individuals of *Rana temporaria* and *Bufo bufo* to age classes, based on their body sizes. Relying on the number of growth rings in phalanges of the animals in each age class, they came to the conclusion that in adult *Rana temporaria* and *Bufo bufo* the first year ring has always been resorbed and that, at the most, only remnants of the resting line of the second year ring are present. However, this conclusion is based on an age-body size relation, which is only valid when all animals of a particular age start at approximately the same body size and grow at a fairly uniform and rapid rate, as already stated by Senning (1940). As body size is

related to phalangeal diameter (chapter 6), the variation in phalangeal diameter at a particular age clearly shows that animals of equal age do not start growing from the same size and thus, that their growth rates vary. This is also supported by the prolonged period of metamorphosis at Feuerloschteich, indicating that the body size of first-year toads at hibernation may vary considerably. Therefore, the method followed by Kleinenberg and Smirina (1969) and by Smirina (1972*a*) might yield unreliable data, at least when used for the populations from Feuerlöschteich and Drie Meertjes. On the other hand, the differences in the degree of resorption of year rings could also be due to the fact that Smirina (1972*a* 1972*b*) and Barbault et al. (1979) analysed different bones (the last but one phalanx of the fourth toe from the hindleg and the femur respectively). If such is the case, this implies that the degree of resorption of year rings varies among phalanges, which is in contradiction with Kleinenberg and Smirina (1969) and Smirina (1972*a*), who stated that in different bones of *Rana temporaria* and *Bufo bufo* the same number of year rings is present.

In the sample of male toads, captured during the spawning period, all males have hibernated at least twice and consequently were at least 2 years old. Further, relative phalangeal growth was found to decrease sharply after the second hibernation which, according to Smirina (1972*a*), occurs only after maturity is reached. This indicates that male toads from both study areas may become sexually mature and sexually active from the age of two. Up to now there are no data available on the age at which female toads become sexually mature. However, the female toads studied in chapter 1 had at least 3 year rings, indicating that they were at least 3 years old. As the degree of resorption in female toads has not been studied yet, it is possible that they attain maturity at an even older age.

The fact that small, late (November) metamorphosed toads from Feuerlöschteich are represented in the sample of adult males to a maximum of only 4, suggests that either most of them do not survive until maturity, or that their number is negligible compared to the number of early metamorphosed toads. In view of the fact that tadpoles from Feuerlöschteich generally metamorphose later and, therefore, have less time for phalangeal growth until hibernation, it is remarkable that the diameter of the first and second year ring in adult males from this site is larger than in those from Drie Meertjes. This implies that the phalangeal growth of young toads from Feuerloschteich is faster from metamorphosis until maturity than that of toads from Drie Meertjes. This fits nicely with the fact that at Feuerloschteich adult body sizes are larger than at Drie

Meertjes (van Gelder, unpublished). Although less probable, the possibility remains that the fast growing young toads from Drie Meertjes, which have reached Feuerlöschteich during their dispersal, spawn here once they have matured, supplying the spawning population from Feuerlöschteich with large toads.

In conclusion it can be stated that the age of male toads from the populations studied can be determined for about 92% by simply counting the number of year rings in cross-sections of a particular phalanx. This percentage can be raised up to 93-98% by adding the number of year rings resorbed. This number could be estimated on basis of the pattern of growth rings in a particular phalanx of the adults and the diaphyseal diameter of this phalanx in first year-toads just before hibernation. The age of the remaining toads may be underestimated by not more than 1 year.

AN IMPROVED METHOD TO ESTIMATE THE NUMBER OF YEAR RINGS RESORBED IN PHALANGES OF BUFO BUFO AND ITS APPLICATION TO POPULATIONS FROM DIFFERENT LATITUDES AND ALTITUDES.

Modified version of:

Hemelaar, A. (1985). An improved method to estimate the number of year rings resorbed in phalanges of Bufo bufo (L.) and its application to populations of different latitudes and altitudes. Amphibia-Reptilia 6: 323-343.

INTRODUCTION

Smirina (1972*a* 1972*b*) found that in phalanges of adult *Bufo bufo* and *Rana temporaria* from a continental climate the first year ring had always been resorbed completely, whereas at best remnants of the second year ring remain present Barbault et al. (1979) reported similar results for the desert species *Bufo pentoni*. In phalanges of male *Bufo bufo* from a temperate oceanic climate (chapter 2) individual variation in the degree of resorption was found, and recently Smirina (1983) confirmed this with respect to *Bufo bufo* from Poland. The phalanges of the toads studied in chapter 2 showed little resorption: in 94% of the toads, the first year ring (or remnants of it) was still present. The number of year rings resorbed in these toads was estimated by a graphical and, if necessary, statistical analysis of the patterns and the diameters of the year rings In all, the degree of resorption of 98% of the toads could be estimated in this way. However, these toads originated from a temperate oceanic climate and it is possible that this method will be less successful when applied to toads from other climatological conditions This chapter shows an improved method to estimate the number of year rings resorbed in phalanges of *Bufo bufo* and its application to toads from different climates.

MATERIAL AND METHODS

Five spawning populations of the common toad *Bufo bufo* were studied at different sites in Europe. The spawning sites and their locations are as follows:

FEUERLÖSCHTEICH (51°45'N,5°58'E), located in Germany close to the Dutch frontier, about 13 km south-east of Nijmegen.

ROELOFSVEN (51°47'N,5°48'E), located in The Netherlands about 8 km south-south-west of Nijmegen.

NAUMAS (43°28'N,2°12'E), located in the South of France, about 65 km south-east of Toulouse. This spawning site is situated at an altitude of about 500 metres at the northern slope of La Montagne Noire.

LITL GJEDDVATNET (63°23'N,10°37'E), located in Central Norway near Trondheim.

LOICHBIEL (46°39'N,8°06'E), located at an altitude of ≈1850 metres in Switzerland near Grindelwald.

The 5 populations are referred to by the first 3 characters of their

Plate 3.1.

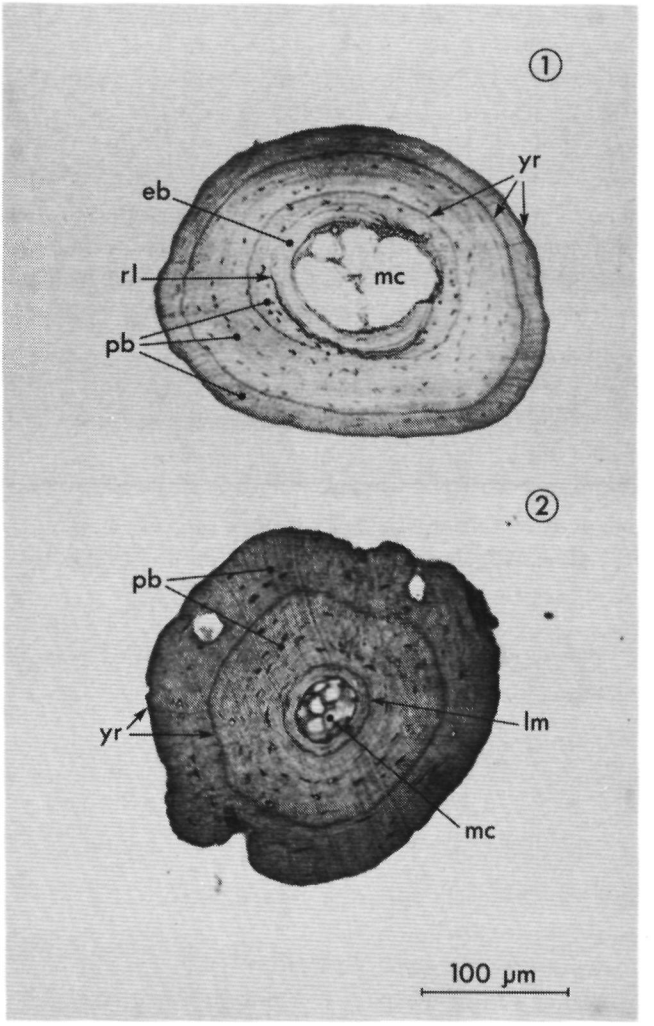


Plate 3.1. *Cross-section of phalanges of male toads from NET. 1. Animal in which periosteal bone has been resorbed. 2. Animal in which no periosteal bone has been resorbed. Legends: mc = marrow cavity, lm = line of metamorphosis, eb = endosteal bone, pb = periosteal bone, yr = resting lines of the successive year rings.*

countries of origin, namely: GER (Germany), NET (Netherlands), FRA (France), NOR (Norway) and SWI (Switzerland). The population of GER was studied in 1980 and 1981, that of NET in 1981. The other populations were studied in 1982. Of the adult toads captured in 1981 and 1982 the third toe of the left forefoot (denoted ft) was amputated. From those captured in 1980, the fourth toe of the right hindleg (denoted ht) was removed. Of each toe only the last but one phalanx was used for this study. The body size (from snout to urostyle) of each toad was measured in mm.

The phalanges were treated and analysed according to the method described in chapter 2. The phalangeal diameters delineated by the resorption line and by each other hematoxylinophilic line were measured (in µm) in the same way as described in chapter 2.

Metamorphosing toads were captured at each site, NET excepted. In addition, at GER (1980), FRA and NOR, first-year toads were captured between metamorphosis and hibernation. Body size was measured in mm and the phalanx (referred to above) of up to 10 toads in each length class (mm) was removed. The phalanges of the first-year toads were cleaned only and their diameters were measured "in toto" under a Zeiss light microscope.

RESULTS

Distinctness of resting lines

In 44 (3%) out of 1471 toads the resting lines were insufficiently distinct to count the year rings, because of poor affinity of the resting lines for hematoxylin. The sections from these toads were not included in further analysis. Table 3.1 shows for each population the number of toads of which the phalanx was sectioned and the number of toads excluded. From the Table it appears that almost half of the toads that had to be excluded originated from NOR. In the remaining toads resting lines were distinct, most in toads from FRA and SWI and least in those from NOR.

Evidence for the line of metamorphosis

Most sections showed the resorption line and endosteal bone, indicating that periosteal bone had been resorbed (Plate 3.1, photo 1). If no endosteal bone was present and, thus, periosteal bone had not been

Table 3.2. *Phalangeal diameter of first-year toads at metamorphosis and just before hibernation, and diameter of the phalanx delineated by lm ($Ø_{lm}$) and that of the first year ring ($Ø_1$) in adult toads. Dates are indicated as decades (I-III) of months; pm = period of metamorphosis, hi = presumed time of hibernation, cd = last capture date of first-year toads, n = number, sd = standard deviation.*

		phalangeal diameter of first-year toads				diameter of $Ø_{lm}$ and $Ø_1$ in adult toads					
site	date	n	mean	sd	range		sex	n	mean	sd	range
NET (ft)	pm: June (II) - July (I)					$Ø_{lm}$	♂♂	37	81	8	62- 96
							♀♀	4	94	8	83-103
	cd:					$Ø_1$	♂♂	37	192	38	151-255
	hi: from November (I)						♀♀	4	179	26	149-202
GER (ht)	pm: July (III) - October (II)	10	84	5	74- 91	$Ø_{lm}$	♂♂	8	74	7	60- 85
							♀♀	12	80	13	59-102
	cd: October (III)	25	168	37	97-237	$Ø_1$	♂♂	8	176	21	136-202
	hi: from November (I)						♀♀	12	180	19	147-204
(ft)	pm: as GER (ht)	11	92	6	79- 99	$Ø_{lm}$	♂♂	37	85	11	67-108
							♀♀	10	83	13	62-108
	cd:					$Ø_1$	♂♂	37	172	18	127-224
	hi: as GER (ht)						♀♀	10	164	11	142-182
FRA (ft)	pm: June (III) - September (I)	16	100	13	76-123	$Ø_{lm}$	♂♂	15	97	12	77-121
							♀♀	2	80	5	76- 83
	cd: September (III)	231	141		90-240	$Ø_1$	♂♂	15	178	20	152-236
	hi: from November ??						♀♀	2	147	4	144-150
NOR (ft)	pm: August (I - II)	13	100	14	81-122	$Ø_{lm}$	♂♂	59	89	8	74-109
							♀♀	12	91	8	74-100
	cd: August (III)	470			106-166	$Ø_1$	♂♂	59	126	13	102-167
	hi: from September (II)						♀♀	12	129	8	116-139
SWI (ft)	pm: September (II)	44	124	11	105-150	$Ø_{lm}$	♂♂	35	77	8	57- 95
							♀♀	9	78	6	70- 87
	cd: September (II)	44	124	11	105-150	$Ø_1$	♂♂	35	106	8	89-134
	hi: from September (III)						♀♀	9	100	8	90-113

Table 3.1. *Number of toads from each population of which the phalanx was sectioned (N) and number (E) and percentage (%) of N that was excluded from further analysis.*

	males			females			total		
site	N	E	%	N	E	%	N	E	%
NET	155	5	3	53	3	6	208	8	4
GER (ht)	132			132			264		
(ft)	241	5	2	122	2	2	363	7	2
FRA	133			95	6	6	228	6	3
NOR	208	13	6	84	8	10	292	21	7
SWI	82	2	2	34			116	2	2
total	951	25	3	520	19	4	1471	44	3

resorbed, a more or less distinct line could be seen quite near the very small marrow cavity (Plate 3.1, photo 2). The phalangeal diameter delineated by this line (denoted $\emptyset_{lm}$) and that delineated by the subsequent line (denoted $\emptyset_1$) are given in Table 3.2. Furthermore, Table 3.2 presents for each population the phalangeal diameter of first-year toads at metamorphosis (NET excepted) and at the last capture date just before hibernation, NET and GER 1981 (ft) excepted. The period of metamorphosis and the (presumed) time of hibernation are also indicated in this Table. Some remarks have to be made with respect to the values of the phalangeal diameter of first-year toads just before hibernation. As the larvae from SWI did metamorphose late -snow already fell a few times during metamorphosis- they are believed to have entered hibernation immediately after metamorphosis. Therefore, the phalangeal diameter at the end of the first year in these young toads from SWI is represented by their phalangeal diameter at metamorphosis At FRA and NOR, the newly metamorphosed toads did have time for growing before hibernation. Their body sizes were measured at various intervals between metamorphosis and hibernation and the diameter of the phalanx of up to 10 toads in each length class (mm) was measured. At the last capture date 231 and 470 first-year toads were captured at FRA and NOR, respectively. Body size at FRA ranges from 9 to 25 mm, with an average of 15 mm. At NOR only the body size of small and large toads were measured, the average body size not being estimated therefore Body size ranges from 12 to 18 mm at this

site. In Table 3.2 the extreme values of the phalangeal diameter just before hibernation in first-year toads from FRA and NOR are represented by the minimum and maximum value of the phalangeal diameter of toads of 9 and 25 mm at FRA, and that of 12 and 18 mm at NOR, respectively. The average phalangeal diameter at FRA is represented by this average value in toads of 15 mm. At the last capture date at GER (1980, ht) only 26 first-year toads were captured. The phalangeal diameters of 25 of them could be measured.

As can be seen in Table 3.2, the phalangeal diameter just before hibernation varies considerably for toads from GER and FRA. Most likely this is caused by the extended period of metamorphosis, which took at least 2 months at these sites, whereas metamorphosis at the other sites occurred within 14 days. Although the time of hibernation in the South of France is unknown, it is believed that the first-year toads from FRA grew for at least another month after the last capture date. Thus, the average phalangeal diameter just before hibernation at FRA might have been considerably underestimated.

Comparing the diameter of the bone delineated by lm ($\emptyset_{lm}$) in the adult toads and the phalangeal diameter of metamorphosing toads, one may say that they match each other closely. Despite the fact that lm is always smaller than the phalangeal diameter of the metamorphosing toads (especially in SWI, see Discussion) it is concluded that lm reflects the period of metamorphosis and, hence, rightly is called the 'line of metamorphosis'. This fits nicely with the fact that $\emptyset_1$ is, though generally larger, comparable to the phalangeal diameter of first-year toads just before hibernation, indicating that $\emptyset_1$ indeed refers to the diameter of the first year ring. Wilcoxon rank-sum tests showed that there is no sexual dimorphism concerning the diameter of the first year ring in these adults (all $P>0.10$, toads from FRA and NET were not tested).

Method to estimate the degree of resorption of year rings

In order to determine the age of an individual we must know the number of year rings resorbed. In toads in which the line of metamorphosis is present no year rings have been resorbed, so that the age of these toads can easily be established by counting the number of year rings. The pattern of year rings in these toads formed the basis for estimating the number of year rings resorbed in the remaining toads. Analysis was carried out for each population and for the two different phalanges (ht and ft), sampled in the population from GER, separately. The method proceeded by

the following steps:

1. Estimating the minimum diameter of the first and second year ring.

1.1. The minimum diameter of the first year ring, $\emptyset_{1min}$, was estimated without subdividing according to sex. $\emptyset_{1min}$ was considered to be the smaller of the values a and b:

a. The average diameter of the first year ring of toads of known age minus twice the standard deviation.

b. The diameter of the smallest year ring measured in toads of known or unknown age.

The diameter of the bone delineated by the resorption line, $\emptyset_{rl}$, in toads of unknown age was then compared with $\emptyset_{1min}$. If $\emptyset_{rl} < \emptyset_{1min}$ it was assumed that in these toads no year ring has been resorbed. Consequently, these toads can now be considered to be of known age too.

1.2. The minimum diameter of the second year ring ($\emptyset_{2min}$) was estimated for both sexes separately. $\emptyset_{2min}$ can be estimated in 3 ways:

a. The average diameter of the second year ring of toads of known age minus twice the standard deviation.

b. The diameter of the smallest second year ring in toads of known and unknown age

c. By considering the relation between $\emptyset_2$ and $\emptyset_1$. Reciprocal and linear relations between $\emptyset_2$ and $\emptyset_1$ of toads of known age (including those, obtained by step 1.1.) were examined. The best fitting relation was obtained by the linear approach:

$$\emptyset_2 = a_1 + b_1\emptyset_1 \quad \text{with residual standard deviation} \quad \sigma_1$$

By entering $\emptyset_{1min}$ in this equation, $\emptyset_{2min}$ can be estimated conform:

$$\emptyset_{2min} = a_1 + b_1\emptyset_{1min} - 2\sigma_1$$

If $\emptyset_2$ was not significantly related to $\emptyset_1$, $\emptyset_{2min}$ was considered the smaller value of 1.2.a and 1.2.b. Otherwise, $\emptyset_{2min}$ estimated with the linear equation and $\emptyset_{1min}$ was preferred, as this value appeared to be the smallest.

The diameter of the first visible year ring in toads of unknown age, indicated as $\emptyset_1$, was then compared with $\emptyset_{2min}$. In toads in

which $\emptyset_1 < \emptyset_{2min}$, $\emptyset_1$ can only refer to the first year ring and, thus, no year ring has been resorbed in these toads. Consequently, these toads were now considered to be of known age too. The number of toads of known age increased by this considerably.

2 Estimating the maximum diameter of the first and second year ring.

Estimation of the maximum diameter of the first and second year ring, $\emptyset_{1max}$ and $\emptyset_{2max}$, was based on the total number of toads of which the age was known so far.

2.1. $\emptyset_{1max}$ was estimated, making no distinction according to sex. $\emptyset_{1max}$ was considered to be the larger of the values a and b:

a. The average diameter of the first year ring plus 3 times the standard deviation. As in the preceding step 1.2. only those toads in which $\emptyset_1 < \emptyset_{2min}$ were added to the number of toads of known age, small $\emptyset_1$ will be present in excess among them. Therefore, the standard deviation was added 3 times.

b The largest $\emptyset_1$ measured in toads of known age.

It must be noted that $\emptyset_{1max}$ should be of about equal size as the maximum phalangeal diameter measured in first-year toads just before hibernation (see Table 3.2).

2 2 $\emptyset_{2max}$ can be calculated either analogous to the estimation of $\emptyset_{1max}$, or by entering $\emptyset_{1max}$ in the equation mentioned above:

$$\emptyset_{2max} = a_1 + b_1\emptyset_{1max} + 2\sigma_1$$

$\emptyset_{2max}$ estimated in this way was preferred, as this value was the larger of the two Only if $\emptyset_2$ was not significantly related to $\emptyset_1$, $\emptyset_{2max}$ was estimated analogous to $\emptyset_{1max}$. $\emptyset_{2max}$ was estimated for both sexes separately.

It is obvious that in toads in which the diameter of the first visible year ring , $\emptyset_1$, exceeds $\emptyset_{1max}$, $\emptyset_1$ cannot refer to the first year ring and, consequently, the first year ring must have been resorbed in these toads. Similarly, if $\emptyset_1$ even exceeds $\emptyset_{2max}$, at least 2 year rings have been resorbed. These toads will be considered later again.

3. Area of overlap between the size distributions of $\emptyset_1$ and $\emptyset_2$.

If $\emptyset_{1max}$ exceeds $\emptyset_{2min}$, the size distributions of the first and second year ring do overlap. Consequently, if in toads of unknown age the first visible year ring $\emptyset_1$ was measured between $\emptyset_{2min}$ and $\emptyset_{1max}$, $\emptyset_1$ might refer to either the first or the second year ring. The diameter

of the second visible year ring, $\emptyset_{i+1}$, in these toads (referring thus to either the second or the third year ring) might be decisive. For this purpose, the linear regression equation between $\emptyset_3$ and $\emptyset_2$ of the total number of toads of known age was computed:

$$\emptyset_3 = a_2 + b_2\emptyset_2 \text{ with residual standard deviation } \sigma_2$$

Knowing the extreme values of $\emptyset_1$ the 95% prediction limits of $\emptyset_2$ at each possible value of $\emptyset_1$ were calculated according to the linear relation between $\emptyset_1$ and $\emptyset_2$ (see Fig 3.1, n=1) In a similar way the 95% prediction limits of $\emptyset_3$ at each possible value of $\emptyset_2$ were calculated (Fig. 3.1, n=2). Fig 3.1 shows for male toads from NOR that these 2 predicted areas overlap from $\emptyset_{2min}$ to $\emptyset_{1max}$, but do not coincide completely So, if in toads in which $\emptyset_1$ was measured between $\emptyset_{2min}$ and $\emptyset_{1max}$, the point ($\emptyset_1$, $\emptyset_{i+1}$) is inside the 95% predicted area of $\emptyset_2$, but outside that of $\emptyset_3$ it was assumed that $\emptyset_{i+1}$ refers to the second year ring and, consequently, $\emptyset_1$ to the first year ring. Hence, no year ring has been resorbed in the toad concerned. If, on the contrary, the point ($\emptyset_1$, $\emptyset_{i+1}$) is outside the 95% predicted area of $\emptyset_2$ but inside that of $\emptyset_3$ (see Fig 3.1b), $\emptyset_{i+1}$ was supposed to refer to the third year ring and $\emptyset_1$ to the second year ring, implying that the first year ring has been resorbed. Only if the point ($\emptyset_1$, $\emptyset_{i+1}$) is within the 95% predicted area of both $\emptyset_2$ and $\emptyset_3$ (Fig 3 1c) it remains uncertain whether $\emptyset_1$ and $\emptyset_{i+1}$ refer to $\emptyset_1$ and $\emptyset_2$, or to $\emptyset_2$ and $\emptyset_3$.

The toads of which the age was still unknown, including those in which at least the first year ring had been resorbed, are analysed further The whole procedure was repeated, except that now, based on the total number of toads of known age, the minimum and maximum size of the subsequent year rings, the linear regression equation between them and the 95% prediction limits were computed. In this way step 1,2 and 3 were repeated until there were no toads left of which the degree of resorption of year rings could be estimated.

The procedure is illustrated diagrammatically in Fig. 3 1 for male toads from NOR. Fig. 3.1 presents, within the extreme values of year ring n concerned (n = 1 to 4), the 95% prediction limits of the subsequent year ring n+1, according to the linear regression equations of the toads of known age. In Fig. 3.1a, the first 4 points are plotted for a toad in which the line of metamorphosis was present and, thus, the age of which was known. Fig. 3 1b shows a male in which the number of year rings resorbed

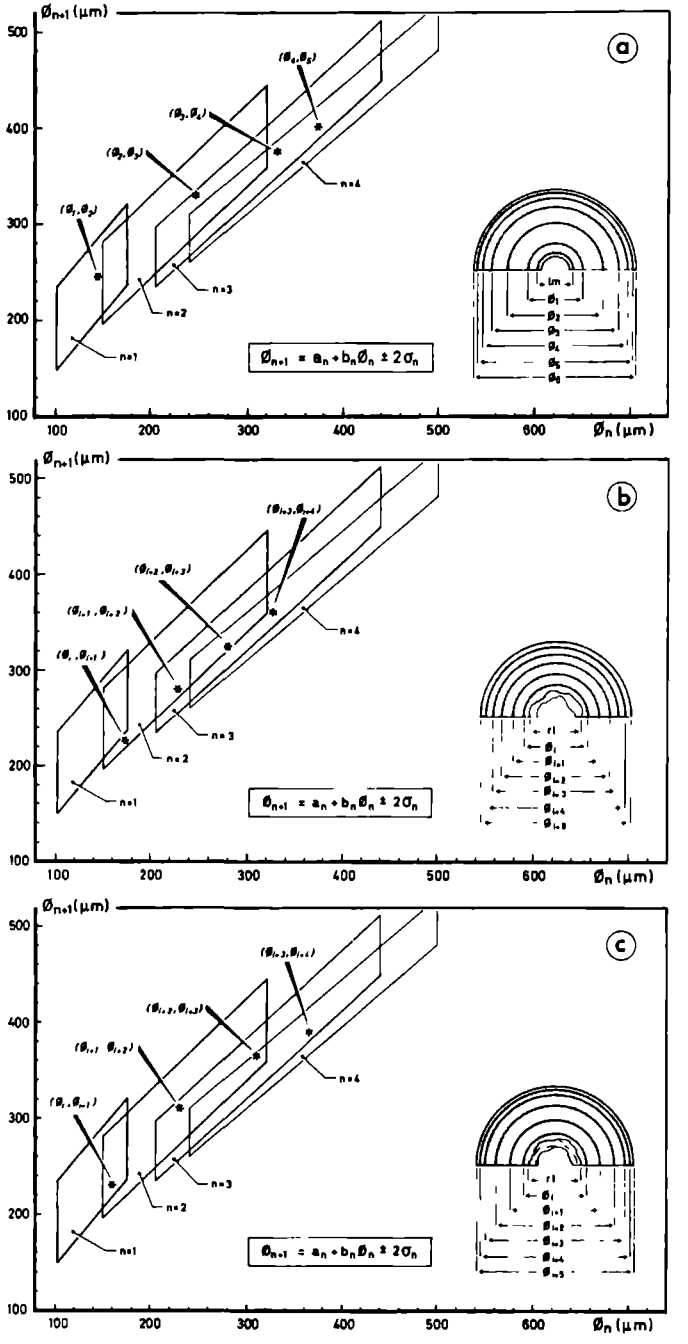

a
b
c
$\emptyset_{n+1}$ (µm)
$\emptyset_n$ (µm)
$\emptyset_{n+1} = a_n + b_n\emptyset_n \pm 2\sigma_n$
n=1
n=2
n=3
n=4
100
200
300
400
500
600

Fig. 3.1. *The extreme values of year ring n (n = 1 to 4) and the 95% prediction limits of year ring n+1 according to the linear equations, in male toads from NOR. Cross-sections of toads are represented diagrammatically and their first 4 points are plotted in the figure.*
a. Animal in which the line of metamorphosis (lm) is present and, thus, no year ring has been resorbed. b. Animal of which could be determined that the first year ring has been resorbed (i = 2). c. Animal in which the number of year rings resorbed could not be estimated (i = 1 or 2). Further explanations, see text.

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was not known initially. However, analysis showed that the point ($\emptyset_i$, $\emptyset_{i+1}$) is only within the predicted area of $\emptyset_3$ and, therefore, $\emptyset_1$ and $\emptyset_{i+1}$ must refer to $\emptyset_2$ and $\emptyset_3$, respectively. Consequently, the first year ring has been resorbed in this toad. In Fig. 3.1c a male toad is shown of which the number of year rings resorbed remained unknown. None or only one year ring might have been resorbed in this toad. Its age can be estimated with an error of 1 year.

In most populations, the size distributions of the subsequent year rings did overlap considerably. Only in the toads from GER the size distributions of the first and second year ring did not overlap (ft) or only slightly (ht). Based on the first visible year ring in these toads, it could immediately be concluded if this year ring referred to the first year ring or not, except for 2 males (GER ht). Step 3 of the analysis concerned only these 2 animals and 4 other males, in which more than one year ring might have been resorbed.

Resorption: sexual dimorphism and variability among populations

The degree of resorption of year rings was assigned to categories, which are defined and illustrated in Fig. 3.2. In Table 3.3, a survey of the results concerning the resorption of year rings in toads from the different populations is given. The number of toads of which the degree of resorption could be estimated was high, ranging from about 82% in male toads from FRA to 100% in male toads from GER (ft). In general, the degree of resorption could be estimated to a greater extent in male toads than in female toads.

In each population there are several toads in which the line of metamorphosis is present, indicating that no periosteal bone has been resorbed (resorption category 0). In the toads assigned to the other

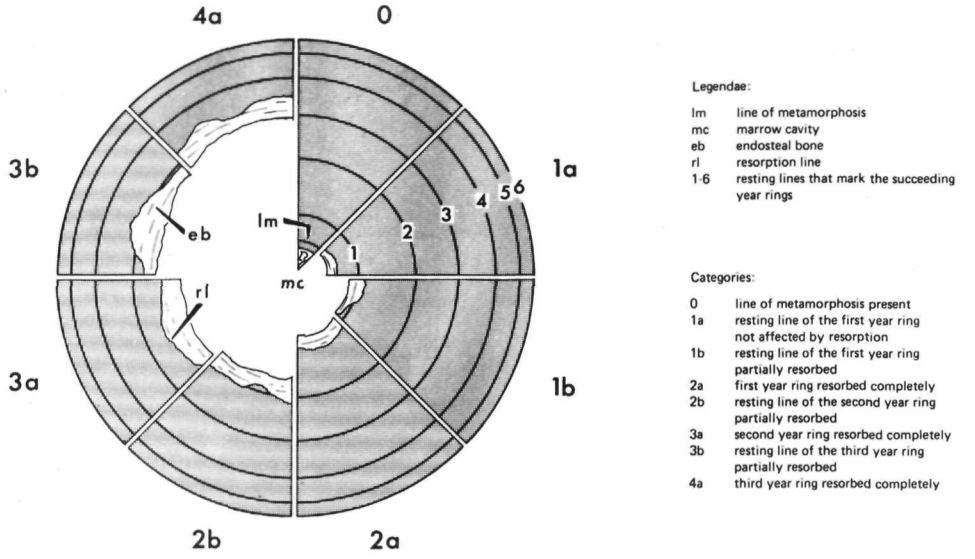

Fig. 3.2. *The degree of resorption of year rings, assigned to resorption categories.*

categories, resorption of periosteal bone occurred to a varying degree. χ^2-test (GER ht excluded) showed that the number of toads in which resorption occurred, differs markedly among the populations ($P<0.001$ for both sexes). However, it is remarkable that in populations in which a high degree of resorption was found (NOR and SWI), yet the number of toads in which no resorption occurred was relatively high. For this reason, resorption category 0 was excluded from the statistics in which differences concerning the degree of resorption were tested. The fact that the body size of toads from resorption category 0 does not differ from that of the other toads (Student's *t*-test, all $P>0.10$) indicates that there is no reason for concern by doing so.

Wilcoxon rank-sum tests showed that in each population resorption of year rings is a more important factor in female toads than in male toads (for *P*-values, see Table 3.3). The fact that in SWI no sexual dimorphism was found might be due to the small number of toads used for this test (44 males and 23 females).

For both sexes, the degree of resorption varies considerably among the populations (Kruskal-Wallis one-way analysis of variance, $P<0.0001$ for both sexes, toads from GER (ht) were not included in this test). Multiple

Table 3.3. *Degree of resorption of year rings in phalanges of Bufo bufo. For resorption category, see Fig. 3.2. Category 'doubts' refers to toads that might be assigned to two categories or more (unknown). Resorption category 0 was excluded from Wilcoxon rank-sum test for sexual differences.*

	NET (ft)		GER (ht)		GER (ft)		FRA (ft)		NOR (ft)		SWI (ft)	
resorption category	♂♂	♀♀	♂♂	♀♀	♂♂	♀♀	♂♂	♀♀	♂♂	♀♀	♂♂	♀♀
0	37	4	8	12	37	10	15	2	59	12	35	9
1a	88	25	52	18	118	25	15	2	6		4	
1b	11	8	62	66	67	54	50	15	40	4	12	5
2a	6	5	7	31	14	29	24	19	46	5	12	4
2b				2		1	5	30	27	38	15	13
3a								6	3	1	1	
3b									3	4		1
Wilcoxon rank-sum test for sex differences	$p < .019$		$p < .000$		$p < .000$		$p < .000$		$p < .000$		$p = .056$	
doubts: 1a or 2a	8	5					4	1				
1b or 2b		2	2				6		1	1		
2a or 3a		1		2			14	5	9	3	1	2
2b or 3b			1	1				7	1	8		
3a or 4a								1				
unknown						1		1				
total	150	50	132	132	236	120	133	89	195	76	80	34

comparison tests (according to Bonferroni, see Dunn 1964, the significance level was lowered to α=0.005) showed that the degree of resorption differs among all populations, except between toads of both sexes from NOR and SWI and between female toads from FRA and SWI. Again the small number of toads from SWI might be the reason Because resorption in toads from NET differs from those from GER (ft), it is obvious that even between populations from the same area (their spawning sites are only about 11 km apart), the degree of resorption of year rings may vary.

The degree of resorption in the phalanx of the forefoot (ft) of male toads from GER is smaller than that in the phalanx of the hindleg (ht), whereas in female toads from this site resorption is the same in both phalanges (Wilcoxon rank-sum tests, P=0 0145 and P=0.369, respectively).

Table 3.5. *Degree of resorption of year rings in phalanges of Bufo bufo of different ages. Age is represented by the number of hibernations. For resorption category, see Fig. 3.2. Trend-test of Terpstra (de Jonge 1960) is used for statistics. Resorption category 0 is excluded from this test.*

| | | RESORPTION CATEGORIES | | |

In addition, in the phalanx of the forefoot the line of metamorphosis was present more frequently than in that of the hind toe.

As can be seen in Table 3.4, resorption of year rings in male toads from NET and GER is low Only a small percentage of the toads is assigned to category 2a or more, indicating that only incidentally the number of year rings present in the periosteal bone does not equal the number of hibernations passed by the animal. However, resorption appeared to be an important factor in female toads from these sites and in toads of both sexes from the other regions

Table 3.4. *Percentage of toads in which at least the first year ring has been resorbed completely (res.cat. ≥ 2a). Only toads of which the degree of resorption of year rings was known are included.*

resorption category ≥ 2a	NET	GER		FRA	NOR	SWI
		ht	ft			
males	4	5	6	27	43	35
females	12	26	25	74	76	56
total %	6	16	12	46	52	41

In Table 3.5 the degree of resorption of year rings in toads of different ages is given. It is remarkable that in each population the youngest female toads are at least 1 year older than the youngest male toads, while resorption in females has progressed further too, as was mentioned above The same was found, when comparing the different populations: with increasing age of the youngest adults, resorption generally has progressed further. Furthermore, if resorption occurs, the degree of resorption does not increase with age in adult toads of both sexes (Trend-test of Terpstra (de Jonge 1960), level of significance α=0.005). Likewise, the presence of the line of metamorphosis does not seem to be restricted to young adults only.

DISCUSSION

In general, relatively few toads were excluded from this study because the resting lines in their bones could not be distinguished sufficiently. However, almost half of them (21) originated from Norway. If distinctness

of resting lines would be positively related with length and severity of the winter, as may be concluded from the study of Klevezal (1973) on mammals, one rather would expect that in toads from the South of France resting lines are poorly visible The contrary is true, the resting lines in the toads from FRA were highly distinct. So, this study provides no evidence that the distinctness of resting lines in the bones of the common toad *Bufo bufo* is a reflection of the length or the severity of the winter.

The line of metamorphosis in adults appeared to be smaller than the phalangeal diameter of the metamorphosing toads, instead of equal-sized. In all probability this is caused by the fact that before measurements were performed, the phalanges of the metamorphosing toads were not treated equally to those of the adult toads (see Method) The same should hold then, of course, with respect to the phalangeal diameter of the first-year toads just before hibernation, in comparison with the diameter of the first year ring in adults. However, the phalangeal diameter of first-year toads just before hibernation is, on the contrary, smaller than the diameter of the first year ring in adults (toads from SWI excepted). Undoubtedly, this is caused by the fact that the last capture date of the young toads did not coincide with the start of the hibernation and, thus, growth may be continued for a while Besides, young toads move away from the pond after metamorphosis and the early metamorphosed and larger toads might have been at greater distance from the pond at the time of capture than the late metamorphosed and smaller ones. Consequently, large toads were less likely to be captured and hence, the sample might be biased to smaller toads. Furthermore, small animals might be more sensitive to unfavourable circumstances than large animals, thereby having less chance to survive hibernation If such is the case, small toads will keep the average phalangeal diameter of first-year toads just before hibernation down, whereas fewer of them are present in the samples of adults. These effects -influence of capture date and composition of the sample- may exceed the effect of the unequal treatment of the phalanx on the measurement itself. For these reasons, bone dimensions measured in first-year toads have to be considered only as an indication of the real dimensions. Despite this, the results do sufficiently support the conclusion that the period of metamorphosis is reflected in the bone as an hematoxylinophilic line.

The extremely high value of the phalangeal diameter of metamorphosing toads from SWI compared to $\emptyset_{lm}$ in the adult toads cannot be explained by the different treatment of the phalanx alone The body size of these young toads was extremely large too, ranging from 11 to 13 mm, whereas

metamorphosing toads ranged from 7 to 12 mm at the other sites. A plausible explanation for these high values might be the relatively cold water at high altitude, especially in 1982, when there was relatively little sunshine during the period of larval development (according to the Meteorologische Zentralanstalt, Zürich). Low water temperatures may result in larger larvae at the time of metamorphosis, as Brand and Grossenbacher (1979) showed for *Rana temporaria* and, to a lesser extent, for *Bufo bufo* from Switzerland.

The line of metamorphosis is of great value, for, if present, it indicates that no periosteal bone has been resorbed and, thus, all initially formed year rings are present. As a matter of fact, it is a prerequisite that in a certain number of the toads from each population the line of metamorphosis is present, in order to establish the degree of resorption in the other toads according to the method presented in this chapter. This condition in the method is the main difference with the method described in chapter 2. However, both methods gave the same results, if applied to toads from GER. Those toads from GER which were sampled in 1980, were analysed by both methods. The conclusions concerning the degree of resorption were identical for each toad. So, for toads from GER -in which both the distributions of the diameters of the first and second year ring overlap only slightly and resorption is relatively low- the less laborious method from chapter 2 will do However, in populations in which these 2 features do not occur together the more laborious method, as presented in this chapter, must be applied.

As the method requires the line of metamorphosis to be present in a certain number of toads, it is very important to choose the phalanx with the slightest resorption. In this respect, the results showed that in female toads from GER resorption is equal in the phalanges studied However, in male toads from this site resorption in the last but one phalanx of the third digit of the forefoot is less than in that of the hindleg. The fact that the forefoot of male toads is used to secure the amplexus might be involved in this. Using the phalanx of the forefoot, the number of male toads in which the line of metamorphosis was present was sufficient to use the method. In toads from FRA and in female toads in general, this number was low, being the main reason that in relatively many of these toads the degree of resorption remained unknown.

There are 2 assumptions underlying this method. First of all, it was assumed that the phalanges of male and female toads grow at the same rate in their first year of life. Without this assumption it would be impossible to

calculate the extreme values of the first year ring in female toads with sufficient reliability. However, the equal diameter of the first year ring in male and female adults in which the line of metamorphosis was present (this chapter) as well as data on phalangeal growth (see chapter 5 and 6) give strong support to this assumption. The second assumption was, that the diameters of the successive year rings do not differ in toads without or with hardly any resorption on the one hand, and those in which resorption has progressed further on the other. This is of fundamental importance, as the extreme values of the first and second year ring are calculated with the values from the former toads, and the other toads are analysed with respect to these values. If the diameters of the year rings would be small in toads in which resorption is low too, this might result in minimum and maximum values of year rings that all are underestimated. With respect to the minimum value, it will not bring about wrong conclusions concerning the degree of resorption. However, a maximum value which is underestimated might certainly do so. For this reason, and for the reason mentioned in the results, the standard deviation was added 3 times to the average diameter of the year ring to be estimated. So, if the toads with which the method starts are not quite representative for the population, the method is corrected for this. If they are representative, maximum values might be over-estimated. However this would only increase the number of toads in which the degree of resorption cannot be estimated. Moreover, in this chapter the body sizes of toads in which no resorption of periosteal bone occurred were not found to be different from those of the other toads. Furthermore, at GER the diameters of the first and second year ring in male toads without or with hardly any resorption did not differ from those of the other toads (see chapter 2). These facts indicate that the second assumption was made correctly.

The fact that the number of year rings resorbed in adults does not increase with age indicates once more that resorption of periosteal bone decreases sharply, once maturity is attained This was already stated in chapter 2 and was mentioned before by Smirina (1972*b*) and Smirina (1972*a* 1983) for *Rana temporaria* and *Bufo bufo*, respectively. Consequently, differences among individuals and among populations with respect to resorption must have arisen before they have attained maturity. This does not agree with Gibbons and McCarthy (1983), who assumed that, if resorption occurs in *Rana temporaria*, it should be most evident in older frogs.

Sexual dimorphism concerning the degree of resorption, also being

reported by Smirina (1983) for *Bufo bufo* from Poland, and differences among populations are obviously related to differences in the age at which maturity is reached The results show a delayed maturation of female toads which goes along with a greater resorption of year rings in female toads than in male toads Furthermore, the trend of greater resorption in populations in which the age of the youngest adults is more advanced, also indicates that resorption increases with increasing age of maturation

In spite of this, a considerable individual variation remains. For example, in a large percentage of the male toads from NOR at least the first year ring had been resorbed completely Occasionally, even the resting line of the third year ring had been partially resorbed. Yet, in many toads the line of metamorphosis was present This individual variation cannot be explained by an individual variation in the age of maturation of male toads from NOR alone As the variation remains within each age class, differences in resorption among cohorts, due to variations in growth rates, cannot be a plausible explanation either This large individual variation in the degree of resorption, even within each age class, is a rather curious phenomenon. Harris and Heany (1970) described bone as a dynamic tissue, which is remodelled continuously. Remodelling of the bone is said to be caused by changes in size and shape (Enlow 1963), changes in function (Harris and Heany 1970) and changes in load on the bone due to the growing animal (Matýas 1955) Shape of the diaphysis and function of the phalanx are not likely to change during growth of a toad, but size of the bone and load on the bone will certainly do. Therefore, it is just the fact that in a number of toads the bone is not remodelled at all, which is most remarkable. Which factors are responsible for this is unknown at this moment.

Because of the large variation in the degree of resorption among populations, even from the same climatological conditions (GER and NET), age determination requires the degree of resorption to be estimated for each population separately. Ignoring resorption of year rings in age determination of *Bufo bufo*, as Gittins et al (1982) did, does not necessarily have to cause serious inaccuracies as is shown for male toads from NET and GER However, the data of male toads from the other sites and those of female toads in general, show that ignoring resorption definitely can bring about many serious errors in age determination (up to 76%). Therefore, individual assessment of the number of year rings resorbed should be preferred The method developed for this has proven to be applicable to populations of *Bufo bufo* which live in a wide range of climatological conditions The disadvantage of the laborious microscopical

measurements, which are required by the method, is amply compensated, by the high reliability of the results. In addition, these microscopical measurements can be used to study other population parameters. For instance, as Gibbons and McCarthy (1983) already have indicated, the yearly increase of the phalangeal diameter informs us about the growth history of each individual. This will be reported in subsequent chapters.

AGE OF BUFO BUFO IN AMPLEXUS OVER THE SPAWNING PERIOD.

Modified version of:
Hemelaar, A.S.M. (1983). Age of Bufo bufo in amplexus over the spawning period. Oikos 40: 1-5.

INTRODUCTION

In the preceding chapters it was shown that in phalanges of *Bufo bufo* from different climates year rings are explicitly present and that the number of year rings resorbed may vary considerably among populations and among individuals from the same population as well as among phalanges of animals Two methods to estimate individually the number of year rings resorbed were described in chapter 2 and 3. The latter has proven to be applicable to populations from different climates, enabling now more detailed investigations on the demography of populations of amphibians, by means of skeletochronology. This chapter, which presents the age of *Bufo bufo* in amplexus over the spawning period is intended to contribute to this.

MATERIAL AND METHODS

Between March 11 and April 11 in 1980, common toads *Bufo bufo*, were captured at Feuerloschteich, a spawning site situated in the Reichswald (see chapter 1). Of these toads the body size was measured from snout to urostyle to the nearest mm and the fourth toe of the right hindleg was removed. During the spawning period 204 pairs were captured of which neither the male nor the female had been captured before in 1980. From this group, 132 pairs were selected for this study. The selected sample reflects both the body size distribution and the date of capture of the 204 pairs.

The age of the toads was determined by skeletochronological analysis of the last but one phalanx from the toe removed, according to the method described in chapter 2 .

RESULTS

The age distribution of the spawning population is shown in Fig. 4.1. The age of 7 toads (3 males and 4 females) out of 264 animals (2.7%) could not be determined exactly, as the number of year rings was doubtful or the degree of resorption could not be estimated. As Fig. 4.1 shows, female toads at the spawning site generally are older than male toads (Wilcoxon rank-sum test, $P<0.0001$) Female toads also appear to pair only from the age of 4 (after 4 hibernations), whereas male toads may pair already from the age of 2.

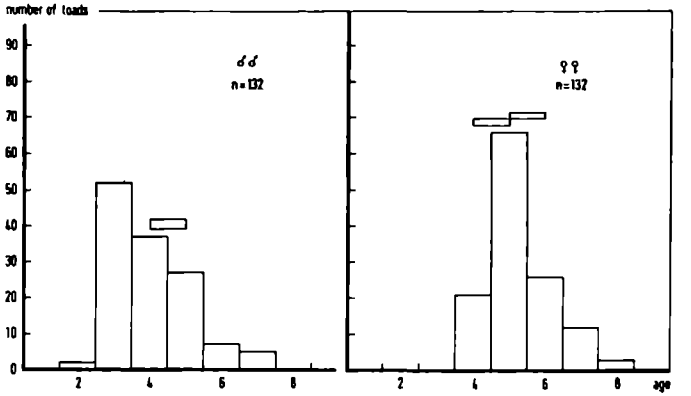

Fig. 4.1. *Age distribution of paired male and female toads from Feuerlöschteich. Blocks overlapping two years refer to toads the age of which could not be assigned to any of these years with certainty.*

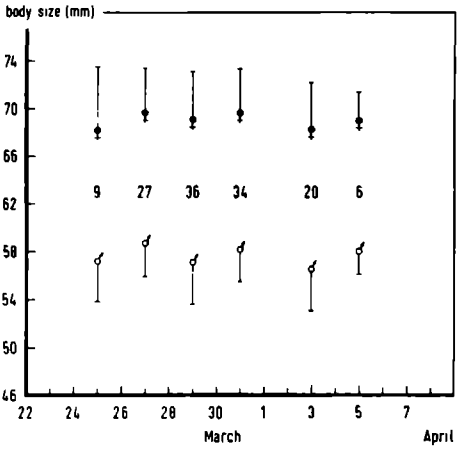

Fig. 4.2. *Average body size and standard deviation of paired toads, captured on different nights during the spawning period. Spawning took place from March 29 until April 5. Numbers refer to the number of toads.*

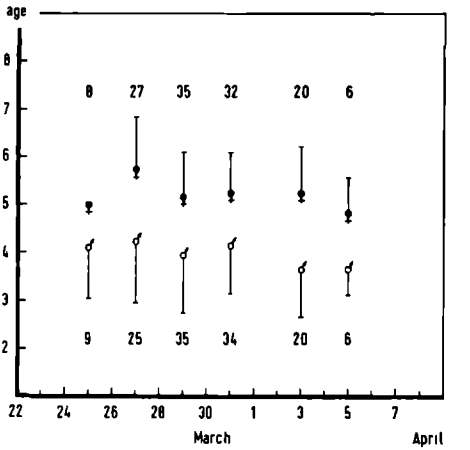

Fig. 4.3. *Average age and standard deviation of paired toads, captured on different nights during the spawning period. Spawning took place from March 29 until April 5. Numbers refer to the number of toads. Only toads the age of which was known with certainty are included.*

Figures 4.2 and 4.3 show the average body size and the average age respectively of the toads captured on different nights during the spawning period. Pairs were found from March 25 until April 5, while the actual spawning took place abundantly from March 29. For both sexes, Wilcoxon rank-sum test showed no difference between the age before and during spawning (both $P>0.10$). The same holds true regarding the body size of male and female toads before and during spawning (Student's t-test, both $P>0.10$).

Table 4.1. *Age composition of pairs of Bufo bufo from Feuerlöschteich.*

male age	female age before spawning					during spawning				
	4	5	6	7	8	4	5	6	7	8
2.....							1			
3.....	1	5	4		1	6	21	6	5	1
4.....		6	3	1		7	11	6	3	
5.....	1	3	2	1		3	11	4		
6.....	1	1		1		1	3			
7.....				1	1		2	1		
	Spearman r = 0.12, p >.50					Spearman r = 0.09, p >.35				

The age composition of the pairs is given in Table 4.1 Only the pairs of which the age of both partners was known are included in the Table. Spearman rank correlation showed no relation between the age of both

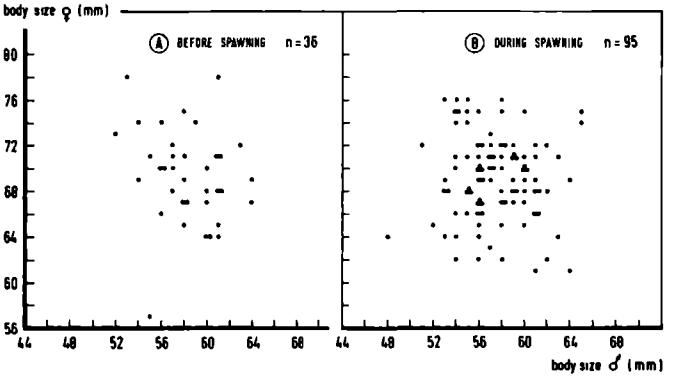

Fig. 4.4. *Body size composition of pairs of Bufo bufo from Feuerlöschteich before (A) and during (B) spawning. Each point refers to one pair.*

partners in the amplexus, neither before nor during spawning. There appears to be no relation either between the body sizes of the partners, neither before spawning had commenced (Fig. 4.4A, Pearson r=0.200, P>0.10), nor during the spawning itself (Fig. 4.4B, Pearson r=0.035, P>0.10).

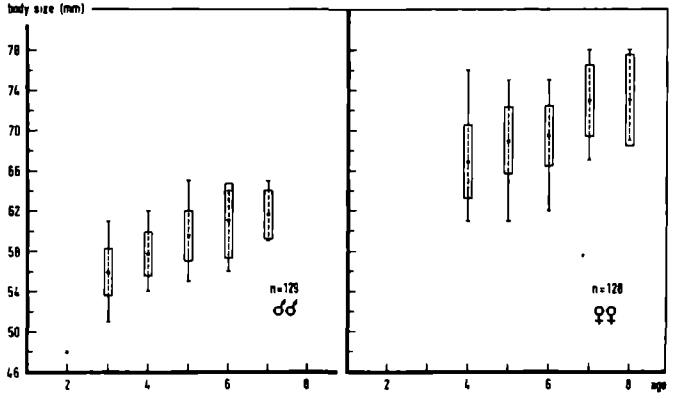

Fig. 4.5. *Average body size, standard deviation (column) and range (bar) of male and female toads of different ages. Only toads the age of which was known with certainty are included.*

Although the body sizes of toads of different ages overlap considerably (Fig. 4.5), toads of both sexes grow larger with age (Trend-test of Terpstra, de Jonge 1960, P<0.01). At each comparable adult age, female toads are larger than male toads (Student's t-test, test of Welch: all P<0.01). Even the youngest females in the spawning population are, on the average, larger than the oldest males.

DISCUSSION

Of more than 97% of the toads examined in this study, the age could be determined exactly. This once more demonstrates the value of the use of skeletochronology in age studies on amphibians.

All paired male toads, except one, were at least 3 years old. This is very remarkable, as in chapter 2 the greater part of a sample of male toads, captured also during the spawning period at Feuerlöschteich, but in another year, was 2 years old. If male toads are indeed sexually mature at

the age of 2, the absence of the second age class in this sample may be caused by poor breeding results of the spawning population two years ago and/or high mortality of this generation during its juvenile and subadult life. However, in chapter 2 the sample was not restricted to paired males only. As at Feuerlöschteich single males outnumber paired males (chapter 5), the sample from chapter 2 will largely consist of single males. Thus, the possibility remains that 2 year old males do migrate to the spawning site, but do not pair before they are 3 years old. On the other hand, if male toads usually mature only from the age of 3 onwards, the 2 years old males reported in chapter 2 may have had such a favourable growth period in their subadult life, that they (or most of them) already attained maturity at the age of 2. If this holds true, the age at which toads become sexually mature is not only controlled genetically, but is also influenced by external factors, and may vary within one population and, perhaps, even within one age class. This would explain the fact that in the age distribution of the females age class 5 outnumbers age class 4, indicating that only a part of the 4 years old females had already reached maturity (apart from the possibility that this age class is less numerous).

Several authors (Frazer 1966, Davies and Halliday 1977 1978 1979, Gittins et al. 1980, van Gelder unpubl.) have mentioned that at the spawning site male *Bufo bufo* outnumber female *Bufo bufo* considerably. Besides the fact that females stay a shorter time at the spawning site and perhaps do not breed every year (Davies and Halliday 1979, Gittins et al. 1980) or may have a higher rate of mortality in their subadult life (Frazer 1966), the fact that male toads evidently attain maturity at a younger age contributes considerably to the imbalance of the sex ratio at the spawning site. The fact that females at the spawning site are older on the average can also be explained by the lower age of maturation of males, rather than by assuming that adult males should have a higher rate of mortality.

Because they are more abundant, male toads have to compete for females (Davies and Halliday 1978 1979). Although large males arrive later at the spawning site than small ones (Gittins et al. 1980), they are more successful in mating since they are able to displace other males from the back of the female (Davies and Halliday 1978 1979). As a result of these displacements one should expect that male toads that eventually spawn are not only larger, but, as body size increases with age, are also older than the male toads that were paired at the start of the spawning period. As neither the average age nor the average body size of paired males from Feuerlöschteich did change during the spawning period, the findings of

Davies and Halliday (1978 1979) and Gittins et al. (1980) cannot be confirmed by the results presented in this chapter.

Davies and Halliday (1977) found no relation between the body size of paired male and female *Bufo bufo* before spawning had commenced. However, in spawning pairs they found a size assortative mate selection. Mina (1974) has noticed also that in *Rana temporaria* from Russia, mate selection occurred by body size. This author therefore assumed that the spawning population tends to a preferential mating of individuals of approximately the same age, although he could not find that mates were clearly selected by age. From the results presented in this chapter it is concluded that *Bufo bufo* from Feuerloschteich does not select mates for spawning by age or body size.

AGE AND GROWTH OF BUFO BUFO FROM A TEMPERATE OCEANIC CLIMATE.

Modified version of:

Hemelaar A (a). Age and growth of Bufo bufo (L.) from a temperate oceanic climate. Submitted for publication.

INTRODUCTION

Until recently, the few studies on amphibian ecology which presented data about the age structure of the population, age at which maturity is reached, growth of different age classes etc, were mainly based on capture-recapture data and on analysis of body size frequency distributions, assuming a relation between body size and age (Heusser 1970, Bell 1977, Daugherty and Sheldon 1982) Although body size increases with age, as shown in chapter 4 for *Bufo bufo* and reported by Hagstrom (1977) for *Triturus vulgaris* and *Triturus cristatus*, several authors (e.g Flindt and Hemmer 1970, Zug and Zug 1979, Hagström 1980, Glandt 1981, Daugherty and Sheldon 1982) mentioned for a wide range of amphibians that the age of adults cannot be estimated from their body size with sufficient reliability. The results from chapter 4 confirms this for *Bufo bufo.*

At present, the year rings found in the skeleton of several amphibians -termed skeletochronology- have proven very useful for individual age determination (see chapter 1, Smirina 1972*a* 1972*b*, Castanet 1975, Smirina and Roček 1976, Hagstrom 1977, Barbault et al. 1979, Francillon 1979, Dolmen 1982, Gibbons and McCarthy 1983 1984, Caetano et al. 1985). Skeletochronology also offers an unique possibility to study the growth history of each animal (chapter 3, Gibbons and McCarthy 1983) Therefore, using skeletochronology in studies on the dynamics of populations of amphibians is very valuable, especially as it does not necessitate the sacrifice of the animals (chapter 1, Smirina 1972*a* 1972*b*)

In this chapter the results of a skeletochronological study on a spawning population of the common toad *Bufo bufo* during two successive years are reported.

MATERIAL AND METHODS

As part of a long term field study on *Bufo bufo*, adult toads were captured in the early springs of 1980 and 1981 at Feuerlöschteich, a spawning site situated about 13 km south-east of Nijmegen. Toe clipping was used to year-mark the toads. In 1980 the fourth toe of the right hindleg (denoted ht) was removed, in 1981 the third toe of the left forefoot (denoted ft). The body size of each toad was measured from snout to urostyle in mm

From the spawning population in 1980, 837 males and 288 females were

captured. The sample selected for skeletochronological analysis was the same as used in chapter 4, i.e. 132 pairs that were not captured before in 1980. The 132 pairs selected reflect both the date of capture and the body size distribution of such paired "new arrivals" (see also chapter 4) In the selected sample 19 males and 23 females were included, which were recaptured from previous years.

A survey of the number of toads captured at the spawning site in 1981 and that of toads selected for skeletochronological analysis is shown in Table 5.1 Out of the total number of 777 males and 391 females, 236 males and 120 females were selected, the sample reflecting the observed overall sex ratio and the body size distribution of both sexes. The selected sample includes 49 males and 10 females which were recaptured from 1980. These numbers reflect the proportion of recaptured toads from 1980 in the sample from 1981, as can be seen in Table 5.1. As toads were not marked individually, it is not sure how many of these selected recaptures are also present in the selected sample of 1980, but it can be calculated that this concerns about 8 out of 49 males (132×49/837) and about 5 out of 10 females (132×10/288). The sample of 236 males further reflects the number of paired and unpaired males in the night that spawning occurred most abundantly as well as the body size distribution of both groups. For the selection of the sample of the females no attention was paid whether the females were paired or not, as all females were supposed to get paired.

Table 5.1 *Survey of the capture data at the spawning site in 1981 and that of the sample selected for skeletochronological analysis.*

	MALES		FEMALES	
	captured	selected	captured	selected
total	777	236	391	120
including recaptures from 1980	166	49	33	10

The age of the toads was determined by skeletochronological analysis of the last but one phalanx of the toe removed, as described in chapter 3. The diameter of each year ring present in the phalanx was measured (see chapter 2) and will be referred to as the phalangeal diameter at a particular age.

In August 1980 about 3000 metamorphosing toads were captured and year-marked by toe clipping. The body size of 21 among them was measured

in a similar way as in the adults. During the last 10 days of October, 26 of these young toads were recaptured in the surroundings of Feuerlöschteich. Their body sizes were measured again.

Statistical tests and data analysis were carried out by means of SAS (1982), a computer system for data analysis. Most of the statistics are described by de Jonge (1960) and by Sokal and Rohlf (1981). The level of significance used was α=0.05, unless mentioned otherwise. When appropriate, α was lowered according to Bonferroni (Dunn 1964).

RESULTS

Age structure of the spawning population

The age structure of the spawning population from Feuerlöschteich in 1980 and 1981 is represented in Fig. 5 1. As can be concluded from the Figure, the age of one male and one female from 1981 was not estimated. In both years female toads at the spawning site are older than male toads (Wilcoxon rank-sum test, α=0 025, both P<0.0001). Females of 2 and 3 years old are lacking in both years. However, 9 out of 23 females from the 1980 sample, which were recaptured from previous years, were 3 years old in the year when they were first captured. Nevertheless, the youngest females at the spawning site are at least one year older than the youngest males. Assuming that the age of the oldest toads at the spawning site reflects their longevity, females may also live longer than males. It is remarkable that the youngest age class present at the spawning site is not the most numerous one, neither for males nor for females.

The age structures in 1980 and 1981 cannot be compared without some comments. In 1980 only paired toads were studied, which were captured over the spawning period, whereas in 1981 paired and unpaired toads were studied, captured during spawning. With respect to the females this makes no difference, as all females at the spawning site were supposed to get paired and to spawn. Concerning the male toads in 1980, the age of paired males captured before spawning had started did not differ from the age of those captured during spawning (see chapter 4) and in 1981 no difference is found between the age of paired and unpaired males during spawning (Wilcoxon rank-sum test, P>0.440). Therefore it is allowed to compare the age structure of the samples from 1980 and 1981.

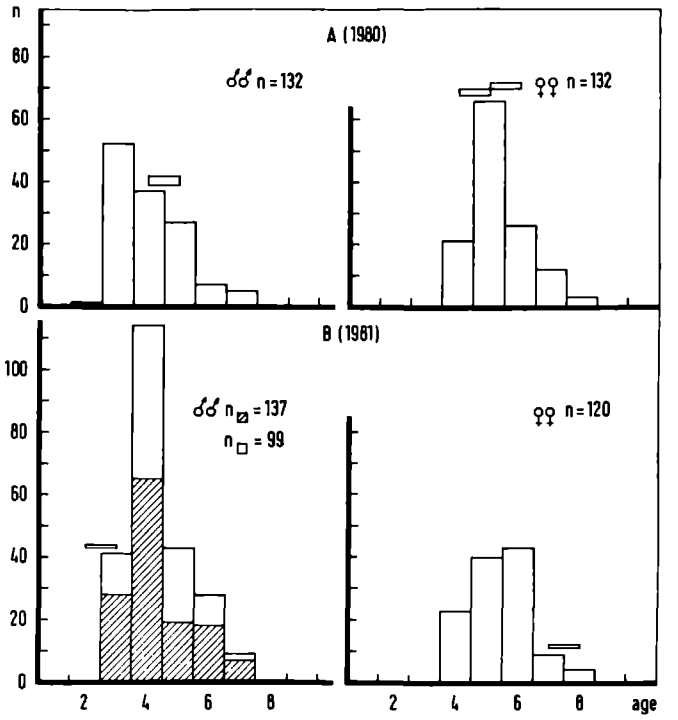

Fig. 5.1. *Age structure of the spawning population from Feuerlöschteich. A. In 1980 (taken from chapter 4). B. In 1981. Shadings refer to unpaired males. Blocks overlapping two years refer to toads the age of which could not be assigned to any of these years with certainty.*

In the age structure of the female toads in 1980 age class 5 outnumbers all the other age classes greatly, whereas in 1981 age class 6 is the most numerous one. Considering that the females from both age classes mentioned belong to the same cohort, the 1975 offspring, the 1975 cohort apparently is very numerous compared to the other cohorts. Although less obvious, the same holds for the age structures of the male toads. Age class 6 in 1981 is relatively more present compared to this age class in 1980 (11.9% versus 5.3%, respectively). However, most notable about the age structures of the males is the great number of 3 year old toads in 1980 and that of 4 year old toads in 1981. These toads belong to the 1977 cohort which evidently is very numerous. Judging from the age structure of the female toads, only a part of the females from the 1977 cohort seems to be present at the

spawning site in 1981.

The validity of these conclusions depends on the extent to which the age structures found in the samples from 1980 and 1981 reflect the age structure of the spawning population in those years. It is possible to discuss this by considering the toads from 1980 that were recaptured in 1981. Let us assume that:

a. the probability to be captured (catchability) is equal among the toads.

b. from a particular age on, all toads of a cohort do migrate to the spawning site.

If both assumptions are valid, this would imply that the age structure of the sample from 1981 should include in each age class the same proportion of toads recaptured from 1980. However, the proportion of toads recaptured from 1980 (R/N) increases with age, as can be seen in Table 5.2. Such might be observed when the catchability of toe clipped toads would increase with age. For several reasons, which will be discussed later, this is not very likely. Consequently, assumption *a* and/or *b* should be revised. Suppose assumption *b* is valid, assumption *a* should be dropped.

Table 5.2. *Number (R) and proportion (R/N) of toads in the sample of 1981, recaptured from 1980. Only toads of known age are included. N = total number of toads in the sample of 1981.*

	MALES			FEMALES		
age	N	R	R/N	N	R	R/N
3	41	3	.073			
4	114	18	.158	23		
5	43	12	.279	40	3	.075
6	28	11	.393	43	2	.047
7	9	5	.556	9	5	.556
8				4		
total	235	49	.209	119	10	.084

Then, the catchability of a toad depends on its age and is reflected by the proportion of recaptured toads (R/N) in each age class in 1981. Judging from R/N in Table 5.2, the catchability generally increases with age. If the catchability of toads of a particular age remains constant throughout the years, it is possible to estimate both the age structure and the size of the

spawning population in both years, by considering R/N, the total number of toads captured in both years and the age structure found in the samples. In Table 5.3 these estimated values are given for male toads only. Although in the estimated age structures the younger age classes are relatively more present compared to those in the age structures found in the samples, both age structures lead to the same conclusions as made before about the relative abundance of the cohorts. However, the estimated age structure rejects its own validity, as it rejects assumption *b* (from a particular age on, all toads of a cohort migrate to the spawning site) under which it was estimated. This appears from the fact that more males of the 1978 cohort were estimated to be present at the spawning site in 1981 (3 years old) than in 1980 (2 years old). Unless the catchability at a particular age varies largely throughout the years, this evidently indicates that not all toads from at least the youngest adult age class do migrate to the spawning site. Consequently, their catchability will be underestimated and therefore their numbers in Table 5.3 will be over-estimated.

Table 5.3. *Estimation of the size of the spawning population of male Bufo bufo and its age structure in 1980 and 1981. P = proportion of recaptured toads (R/N) from Table 5.2, S = number of toads of known age in the samples, E = estimated number of toads present at the spawning site.*

		1980		1981	
age	P	S	E=(S/P)×(837/129)	S	E=(S/P)×(777/235)
2	.073	1	89		< 45
3	.158	52	2135	41	858
4	.279	37	860	114	1351
5	.393	27	446	43	362
6	.556	7	82	28	167
7	< 1.0	5	> 32	9	> 30
total		129	> 3644	235	≈ 2813

Since assumption *b* is not valid, assumption *a* might be valid or not. Suppose assumption *a* is valid (the catchability is equal among toads), the age structure of the sample correctly reflects the age structure of the spawning population. Suppose assumption *a* is not valid, the age structure of the spawning population will be intermediate between that of the sample

(Fig. 5.1) and the estimated one (Table 5.3). For, in the sample the younger age classes will then be underestimated, as unequal catchability was not considered, whereas in the estimated age structure they will be over-estimated, as partial breeding migration was not considered. Whatever the real age structure precisely is, it will lead to the same conclusions as already were made about the relative abundance of the cohorts.

Body size and body growth

Figure 5.2 represents the body size distribution of the toads from the spawning population in 1980 and 1981. In both years female toads are larger than male toads (t-test, α=0.025, both P<0.0001). The average difference is almost 12 mm. With reference to the unequal composition of the samples from 1980 and 1981, it is noticed that (analogous to the age) neither the body sizes of paired toads captured before and during spawning in 1980 were different (see chapter 4), nor are the body sizes of paired

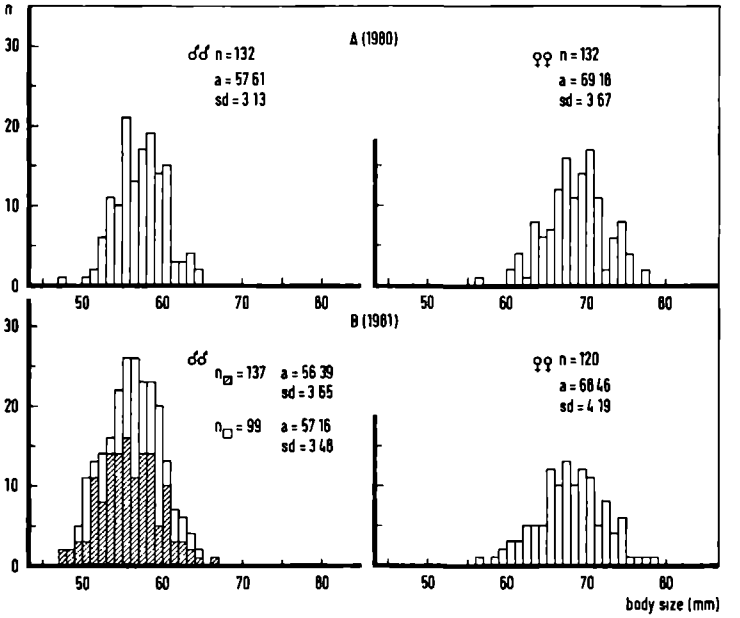

Fig. 5.2. *Body size distribution of male and female toads from Feuerlöschteich in 1980 (A) and in 1981 (B). Shadings refer to unpaired males. n = number, a = average body size, sd = standard deviation.*

and unpaired males in 1981 (Fig. 2B, t-test, $P>0.100$). Consequently, the body sizes of the toads in 1980 and 1981 may be compared. There appeared to be no difference in female body size in both years, whereas in 1980 males were larger than in 1981 (t-test, $\alpha=0.025$, $P>0.157$ and $P<0.019$, respectively).

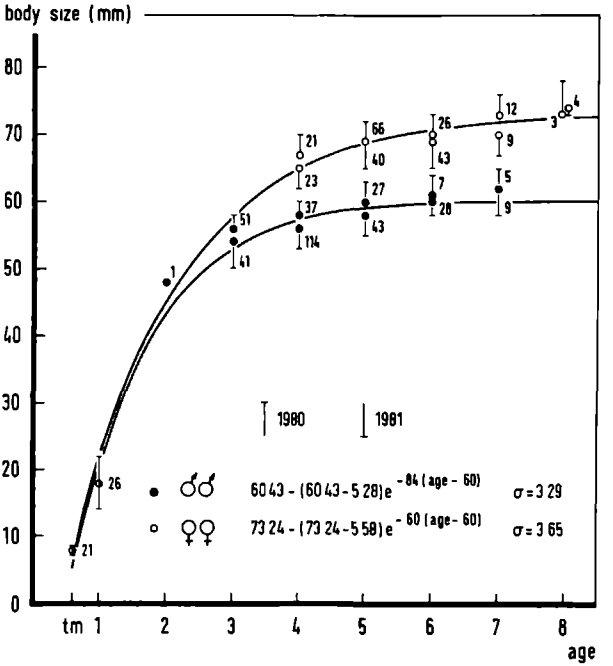

Fig. 5.3. *Average body size and standard deviation (bars) of Bufo bufo from Feuerlöschteich at different ages. Time interval between adjacent ages is the annual growing season. The data of the adults from 1980 are taken from chapter 4. The body growth curves and their equations are also included.* t_0 *= age at metamorphosis.*

The average body size of toads of different ages is shown in Fig. 5.3. Three-way analysis of variance for unequal sample sizes was used to analyse the data of the adults. The main effects of sex, age and year-of-capture on body size were found to be highly significant (all $P<0.009$). These main effects were investigated further with t-tests at lowered levels of significance. The effect of sex on body size is caused by the fact that female toads are larger than male toads at each comparable

adult age (α=0 0063, all P<0 0001). To locate the age effect, comparison tests were carried out stepwise. From those it appeared that in both years 3 year old males are smaller than 4 year old males (α=0.0125, P<0.0001) and 4 year old toads of both sexes are smaller than 5 year ones (α=0 0063, P<0.002). This indicates that in general body size increases significantly until the age of 5. Relying on this one would expect that 3 and 4 year old toads in 1980 (the 1977 and 1976 cohorts) would grow significantly, resulting in a larger body size in 1981, being then 4 and 5 years old, respectively However, the body sizes of neither of these cohorts did grow significantly in 1980 (t-tests, α=0.017, P>0 450), implying that in 1980 toads grew less than normal. The little growth during the growing season in 1980 is supported by the results of the investigation of the year-of-capture effect in males. In 1981 males of 3, 4 and 5 years old were smaller than those of comparable age in 1980 (α=0.01, P<0.01). However, the body sizes of female toads at equal age in 1980 and 1981 did not differ (α=0.010, P=0.040 at age 4 and P>0 85 at age 5).

To describe body growth after metamorphosis in general, the data of the adults from both years were combined and those of the juveniles were included. The age at metamorphosis (t_0) represents the proportion of the growing season already passed when metamorphosis occurs. The body size at t_0 and at age = 1 is reflected by the body size of metamorphosing toads and that of first-year toads just before hibernation (captured over the last ten days of October 1980), respectively. These juveniles were not divided according to sex. The data were fitted to the model of von Bertalanffy (1938) for linear growth·

$$S_t=S_m-(S_m-S_0)e^{-k(t-t_0)}$$ residual standard deviation σ

In this equation t is expressed as the number of growing seasons experienced (= age), parameter S_m represents the estimated average maximum body size that can be reached and S_0 the estimated average body size at metamorphosis ($t=t_0$). The growth coefficient k defines the shape of the curve, thus, when S_m will be reached. In Fig. 5.3 the growth curves are drawn for male and female toads. According to the equations, both sexes reach the same body size in their early life. Already during the second growing season, however, body growth of males seems to slow down more rapidly than that of females, resulting in the above-mentioned sexual dimorphism concerning body size from maturity on The difference in the estimated maximum body size of male and female toads amounts to 12.74 mm.

Phalangeal diameter and phalangeal growth

The phalangeal diameter at different ages is shown in Fig 5 4 The phalangeal diameter at metamorphosis is represented by the diameter of the line of metamorphosis, which was measured in the phalanx of several adults (see chapter 3). Overall analysis of the data with a three-way analysis of variance for unequal sample sizes showed significant main effects of the type of the phalanx, sex and age on phalangeal diameter (all $P<0.0001$). Further investigation of each of these main effects at $\alpha=0.005$ revealed that in male toads the phalanx from the forefoot is smaller than that from the hindleg from the age of 2 (t-tests, from age 2 all $P<0.0001$), whereas both phalanges in female toads have equal diameters at each comparable age (t-tests, $P=0.0067$ at age 1, other $P>0.360$). Sexual dimorphism is apparent from age 3 in the phalanx from the hindleg (t-tests, $P<0$ 002 from age 3) and already from age 1 in that from the forefoot (t-tests, from age 1 all $P<0.0001$). In males as well as in females the phalangeal diameter increases significantly until age 5 (t-tests for matched pairs, until age 5 all $P<0.002$).

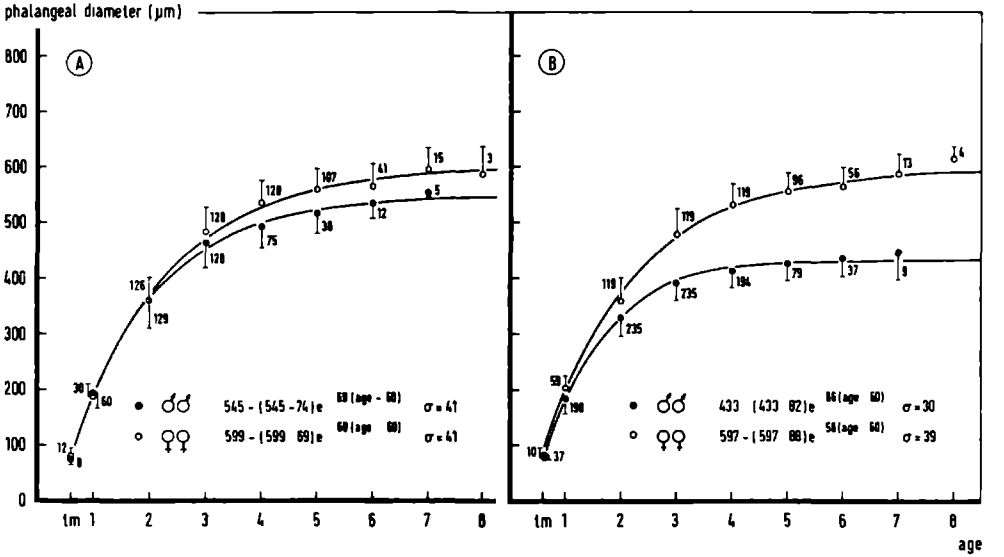

Fig. 5.4. *Average phalangeal diameter and standard deviation (bars) in Bufo bufo from Feuerlöschteich at different ages. Time interval between adjacent ages is the annual growing season. A. Phalanx from the hindleg, sampled in 1980. B. Phalanx from the forefoot, sampled in 1981. The phalangeal growth curves and their equations are also included.*

Analogous to body growth, phalangeal growth can be described by a negative exponential regression curve. The phalangeal growth curves are given in Fig. 5.4, for both sexes separately. Just like body growth, phalangeal growth decreases in male toads at a younger age and more rapidly than in female toads. The coefficient k for body growth and phalangeal growth and their 95% confidence limits are given in Table 5.4. The growth coefficients in female toads do not differ within their 95% confidence limits. In male toads the 95% confidence limits of the growth coefficient of the phalanx from the hindleg (ht) do not overlap with those of the other growth coefficients in males, whereas they only just overlap with those of the comparable growth coefficient in female toads. The other comparable growth coefficients in both sexes do not overlap within their 95% confidence limits.

Table 5.4. *Coefficient k for body growth and phalangeal growth and the 95% confidence limits (c.l.) in male and female toads from Feuerlöschteich. Growth coefficients are taken from the growth equations in Fig. 5.3 and Fig. 5.4.*

	MALES		FEMALES	
	k	95% c.l.	k	95% c.l.
body	0.84	0.79 - 0.90	0.60	0.54 - 0.66
bone ht	0.69	0.63 - 0.75	0.60	0.56 - 0.64
bone ft	0.86	0.82 - 0.91	0.58	0.54 - 0.62

The phalangeal growth history of toads from each cohort is given in Fig. 5.5. To compare at each age the annual phalangeal growth among the cohorts, one-way analyses of variance were used, for both phalanges and sexes separately. For male toads differences in phalangeal growth among the cohorts were found from age 1 to age 2 (both phalanges, both $P<0.02$) and from age 2 to age 3 (ft, $P<0.013$), for female toads from age 2 until age 5 (both phalanges, all $P<0.04$). Multiple comparison tests at $\alpha=0.003$, to locate the differences, showed each time that in 1979 phalangeal diameter increased more, in 1980, 1976 and especially in 1977 phalangeal diameter increased less than normal. The results permit to list the years in order of decreasing phalangeal growth as follows:

1979 > (1978 1975 1974) > 1980 > (1976 1977)

If no significant differences were found among two or more years no sign is placed in the series above. Apparently, the environmental conditions in 1979 were favourable for the growth of young toads, whereas in 1976 and 1977 they were not.

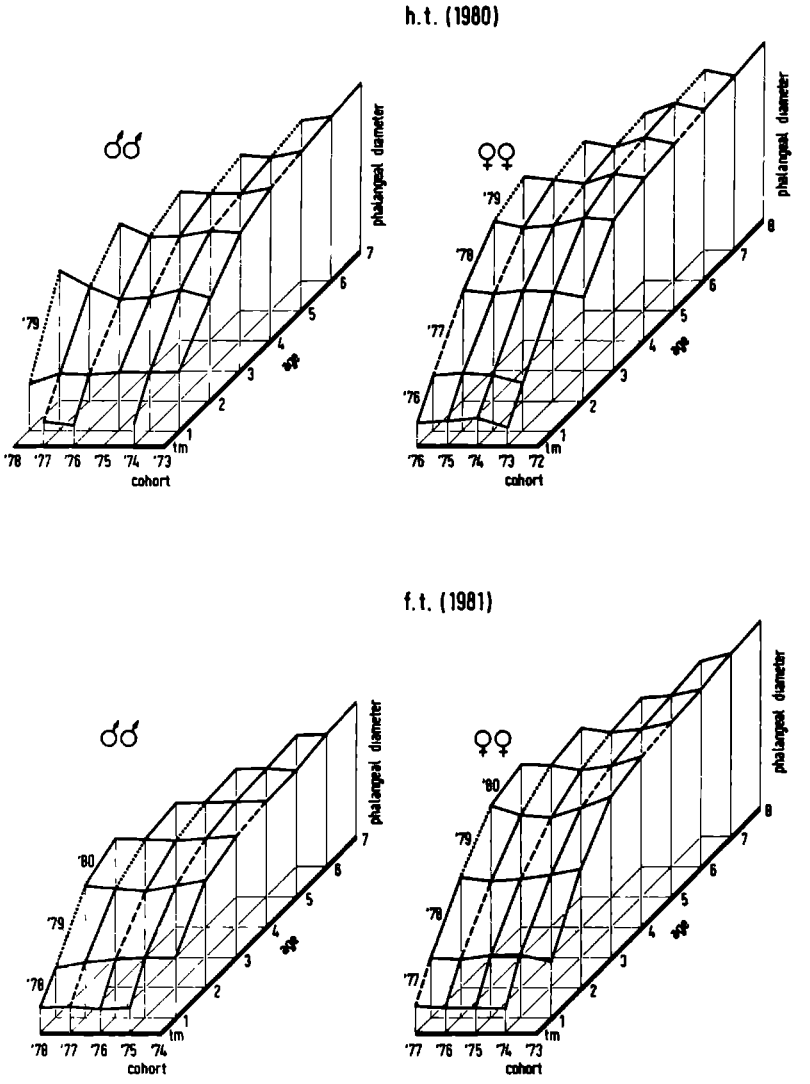

Fig. 5.5. *Phalangeal growth of each cohort. Broken and dotted lines refer to the phalangeal growth in 1977 and in 1979, respectively.*

Gittins (1983*a*) reported that only a small number of female *Bufo bufo* present at the spawning site did not breed, whereas Davies and Halliday (1978) and Wisniewski et al. (1980) assumed that all females present will spawn. The latter holds also for female toads from Feuerloschteich (van Gelder, personal communication). However, approximately only half of the males from this population will spawn, as they outnumber females by about two to one.

In this study it was supposed that males which were paired in the night in which spawning occurred most abundantly, indeed spawned, whereas males which were single that night did not. Although the average body size of spawning males in 1981 was larger than that of non-spawning males, the difference between these body sizes was not significant. This does not agree with Davies and Halliday (1977, 1979), Gittins et al. (1980) and Reading and Clarke (1983), who all found that spawning male *Bufo bufo* were larger than non-spawning males. Larger males are said to be more successful in holding and taking over a female in amplexus (Davies and Halliday 1977, 1978) and, consequently, large males should have a better chance to mate in populations in which male-male interactions occur by way of fights for females If such is the case, it is conceivable that a higher sex ratio in a given spawning population causes more fights among males, thus causing the advantage of a larger body size to become more effective. Sullivan (1984) indeed mentioned a relation between the intensity of sexual selection and the operational sex ratio Against this background, the contradictory results concerning the body size of spawning males may be the result of the lower sex ratio in the spawning population from Feuerlöschteich in 1981 (overall sex ratio=1.99), compared to that in the populations studied by Davies and Halliday (1979), Gittins et al. (1980), Gittins (1983*a*, 1983*b*) and Reading and Clarke (1983), the overall sex ratio ranging here from 2.73 to 5. However, it is also possible that the relatively small number of toads, used for the statistical test in this study, prevented a possible difference between the body sizes of spawning and non-spawning males to become manifest.

It may be questioned whether the age structure of the samples from 1980 and 1981 indeed reflects the age structure of the spawning population in those years. The different age structures in 1980 and 1981, the fact that the youngest age class is not the most frequent one and that in 1981 the proportion of toads recaptured from 1980 increased with age, gave reason

for this doubt Three factors -each affecting more or less the validity of the age structure found- can explain these observations.

First of all, it is possible that the catchability of toe clipped toads increases with age in the way as reflected by the proportion of recaptured toads in each age class. If this is true, it would impair the validity of the age structure of the sample. Toe clipping has become a common technique for marking Anura and it generally proceeds quite satisfactory without apparent harm to the animal (Martof 1953, Moore 1954, Heusser 1968*a*), although Collier (1970) reported inflamed and swollen digits in toe clipped *Bufo bufo*. Clarke (1972) found that the catchability of *Bufo woodhousei fowleri* decreased as the number of toes removed increased, small and large toads being equally affected. Inflamed and swollen digits were only rarely encountered in toads from Feuerlöschteich. Furthermore, the number of toes removed from a particular toad in this study was mostly one and, if more, not related to the body size and the age of the animal. So, if there would be any effect of toe clipping on the catchability of *Bufo bufo* from Feuerloschteich at all, the effect will be equal at each age and, thus, will not impair the validity of the age structure of the sample.

Second, the catchability of toads, marked or not, may increase with age. An unequal catchability like this can explain the observed imbalance in the proportion of recaptured toads in each age class and it certainly would cause the age structure of the sample to deviate from that of the spawning population Concerning the catchability of *Bufo bufo*, Gittins (1983*b*) stated that the capture system he used -toads were collected on roads surrounding their spawning site- is unselective for age and therefore the catchability should be equal for each individual. The toads from Feuerloschteich were mainly captured from the water with a landing-net. Van Gelder and Rijsdijk (manuscript in prep) have studied this population in the same way and they found that large toads were recaptured more frequently within the spawning period than smaller ones. This also appears from the study of Clarke (1972) on *Bufo woodhousei fowleri* and that of Gatz (1981) on *Bufo americanus*. It indicates that the catchability of toads increases as body size and, thus, age increases. Whether this unequal catchability is caused by an age or size selective capture system is not clear Behavioural aspects, for instance the display of toads at the spawning site or the length of their stay (as assumed by Gatz 1981), might change with size and age and, thus, might also be responsible for unequal catchability In conclusion, unequal catchability indeed is quite probable for toads from the spawning population from Feuerlöschteich. However, the

fact that the youngest age class is not the most frequent one cannot be explained by this alone, as the catchability of the youngest toads should then be extremely low compared to that of the older toads.

The third factor, which can explain the age structure found in the samples, is the possibility that not all toads reach maturity at the same age, causing a partial breeding migration of toads from especially the younger age classes. In this case, the number of adults in a cohort will increase for some years, whereas on the other hand mortality lowers the number of toads in a cohort throughout the years, resulting in an age structure of the spawning population as observed in the samples. Once they have reached maturity, male and female toads from Feuerlöschteich are believed to migrate to the spawning site each year. Although Heusser (1968*a*) and Daugherty and Sheldon (1982) mentioned that most females of *Bufo bufo* and *Ascaphus truei* spawned in alternate years, the work of Frazer (1966), Hede (1976), Wisniewski et al. (1980) and my results clearly showed that a considerable number of female *Bufo bufo* returned in consecutive years. Jørgensen et al. (1979) and Gittins (1983b) even stated that the entire population of sexually mature toads migrate to the spawning site each year. Therefore, it is likely that at Feuerlöschteich partial breeding migration does not have to be considered for the older age classes. A partial breeding migration of only the younger age classes also explains the imbalance in the proportion of recaptured toads in the adult age classes, since the proportion of adult recruits will decrease with age, causing the proportion of recaptured adults to increase with age.

Summarizing, the catchability of young toads is lower than that of old ones. Consequently, in the age structure of the sample the number of young toads present at the spawning site will be underestimated when their lower catchability is not taken into account. On the other hand, their numbers will be over-estimated in the age structure estimated on the basis of the proportion of recaptured toads (R/N) when partial breeding migration is ignored (as shown in the results for male toads). The effect of partial breeding migration of toads from the younger age classes seems to exceed largely the effect of their lower catchability. Therefore, the age structure of the younger age classes in the spawning population is quite well reflected by that of the sample, for the older age classes the estimated age structure is probably more valid. However, it must be kept in mind that the size and the age structure of the spawning population in 1981 was estimated under the assumption that the catchability at a particular age remains constant throughout the years. Unfortunately, this can be verified

only, when the population is studied for several consecutive years.

As already was suggested, not all members of a cohort attain maturity at the same age This suggestion was also made by Gittins et al. (1982) and Smirina (1983) for *Bufo bufo*, by Flindt and Hemmer (1970) for *Bufo calamita* and *Bufo viridis*, by Bell (1977) for *Triturus vulgaris* and by Bruneau and Magnin (1980) for *Rana catesbeiana*. It is generally known that amphibians must reach a minimum body size before they become sexually mature (e.g. Ryan 1953, Flindt and Hemmer 1970, Hede 1976, Hede and Jørgensen 1978, Zug and Zug 1979, Glandt 1981). According to Jørgensen and Billeter (1982) body size is a more important factor than age in determining the onset of sexual maturation. Variations in the individual growth rates in a cohort may then cause a variable age at which this minimum body size is reached.

In the spawning population from Feuerlöschteich 95% of the male and female toads were larger than 51mm and 62mm, respectively. These body sizes can be taken as the minimum body size at maturation. As at each age the body sizes of toads are normally distributed, the standard normal deviate (snd) of the minimum body size at maturation can be calculated at each age, according to:

$$snd_t=(S_{mat}-S_t)/\sigma_t$$

in which S_{mat} = minimum body size at maturation
S_t = average body size at age t
σ_t = standard deviation of the body size at age t

Then, the probability *P* of snd_t reflects the percentage of toads of age t which has reached S_{mat}, that is the maximal percentage of sexually mature toads of that age This percentage can be estimated in the same way with the phalangeal diameters Table 5.5 shows that the maximal proportions of sexually mature toads in each age class estimated with S_{mat} for body size and for phalangeal diameters correspond quite well, although they vary in the youngest adult age class for both sexes. With these values, the catchability at a particular age, reflected by the proportion of recaptured toads in the age classes, may be corrected. Using the corrected values, the number of toads of each cohort which migrate to the spawning site and, thus, the real age structure of the spawning population can be estimated. However, by variations in the environmental conditions the proportion of sexually mature toads in a particular cohort will vary throughout the years. Indeed, 3 year old females were absent in both years, although they can

Table 5.5. *Maximal proportion of sexually mature toads in each age class, as estimated with S_{mat} for body size (mm) and phalangeal diameters (μm).*

	S_{mat}	AGE CLASSES			
		2	3	4	5
MALES					
body	51	0.01	0.75	>0.95	
phalanx ht	440	0.03	0.74	0.95	
phalanx ft	360	0.14	0.83	>0.95	
FEMALES					
body	62		0.12	0.78	>0.95
phalanx ht	493		0.31	0.82	>0.95
phalanx ft	490		0.30	0.82	>0.95

migrate to the spawning site at this age, as indicated by the fact that 9 out of 23 females, recaptured in 1980 from previous years, were 3 years old when captured for the first time. In this respect, it is also noted that the equation for body growth was estimated with the body sizes from only two cohorts in each age class. When the body sizes in especially the younger age classes deviate from the average body size at those ages (as presumed in 1981), the estimated growth equations and, consequently, the estimated proportions in Table 5.5 do not reflect the average situation. This problem is reduced when estimating the phalangeal growth equations, for at each age phalangeal diameters are available of all cohorts present at the spawning site.

The observed sexual dimorphism concerning the age of maturation is mentioned before for various species of amphibians (Smirina 1972*a* 1983, Howard 1980, Berven 1981, Gittins et al. 1982, Daugherty and Sheldon 1982). In all probability delayed maturation of female toads is responsible for the observed sexual dimorphism concerning body size and phalangeal diameter. When toads prepare themselves for their first spawning, energy of ingested food should be used for maintenance, growth and reproduction (Hede and Jørgensen 1978). So, concerning the population from Feuerlöschteich, less energy will be available for growth from the age of 2 for most male toads, whereas female toads may continue rapid growth for at least another year. As a result, sexual dimorphism concerning body size and phalangeal diameter develops before both sexes are sexually mature. This is in accordance with Jørgensen (pers. comm.), who found that the

growth rate of male and female *Bufo bufo* from Denmark begins to diverge at a body size of 35 mm, when the sexes cannot be distinguished yet on the basis of external characteristics.

The delayed maturation and continued growth of female toads is reflected by the growth coefficient k found in the negative exponential growth curves: a lower value of k indicates a later and less drastic decrease of growth and, apparently, a delayed maturation. In this respect the growth coefficient of the phalanx from the hindleg in male toads is exceptional, as it is comparable to that in female toads, whereas it differs from the other growth coefficients in males. This can be explained if the function of the digits from those limbs is considered. In all probability, the function of the digits from the hindleg is equal in both sexes, the function of those from the forefoot, however, is not. In addition to the normal function, male toads use the inner two digits from the forefoot to hold a female in amplexus. According to Matýas (1955) and Harris and Heany (1970), a different function will place a different mechanical load on the bone and, therefore, growth will also be different. Judging from the comparable growth coefficients of both phalanges in females and that of the phalanx from the hindleg in males, the mechanical load on these bones does not differ. Apparently, the special function of the digits from the forefoot in male toads causes only the growth coefficient of the phalanx from the forefoot in male toads to be different from that of the other phalanges. This agrees with Oka et al. (1984), who found sexual dimorphism only in those muscles from the forelimb of *Bufo japonicus* which are involved in performing the amplexus. As the growth coefficient of the phalanx from the forefoot does not differ from that of the body, sexual dimorphism concerning body growth is best reflected by the growth of this phalanx.

Although in both sexes body and phalanges grow significantly until the fifth hibernation, both of them show considerable overlap between the adjacent age classes. From this it becomes obvious that estimating the age of adult toads from size-frequency distributions might bring about many errors, at least in populations in which growth proceeds comparably.

Environmental conditions apparently influence phalangeal growth. Considered together with 1977 and 1976, which appeared to be years with extremely little growth, 1980 was also a year in which phalangeal growth was less than normal. Judging from the data on body size, 1980 was not favourable for body growth either. This probably explains the fact that the average body size of males in the sample from 1981 was smaller than that of males in the sample of 1980. Temperature, precipitation and humidity

during the period of active feeding are presumably the most important weather conditions, which may influence the activity of *Bufo bufo* as well as the availibility of its food, thereby influencing the growth of the toad. A very hot and dry summer like in 1976 (KNMI, Weather reports, station nr. 380) seems to reduce the activity of toads, whereas probably less food is available in extremely wet and cold summers. However, the series of years with decreasing growth, found in the results, cannot be explained by the weather conditions in those years with respect to the 3 factors mentioned (KNMI, Weather reports, station nr. 380). Either the weather reports concerned do not reflect well the conditions in the microhabitat of *Bufo bufo*, or other hitherto unknown factors are of major importance for growth.

As most amphibians are difficult to capture in their subadult life, data on growth of subadults in nature were usually lacking In addition, information about individual body growth of adults require many years of capture and recapture, which is very time-consuming Fortunately, skeletochronology offers the possibility to obtain information about the growth of each animal from just one sample, as in the skeleton the entire growth history of the animal is recorded Therefore, skeletochronology will be involved more and more in ecological studies on amphibians.

AGE, GROWTH AND OTHER POPULATION CHARACTERISTICS OF BUFO BUFO FROM DIFFERENT LATITUDES AND ALTITUDES.

Modified version of:

Hemelaar A (b). Age, growth and other population characteristics of Bufo bufo (L.) from different latitudes and altitudes. Submitted for publication.

INTRODUCTION

The geographical distribution of the common toad *Bufo bufo bufo* (L.) extends from North-West Africa over entire Europe (except for the extreme North, Ireland and the Mediterranean isles) to Japan (Angel 1946, Mertens 1947). However, in the South of Europe other varieties occur, including *Bufo bufo spinosus* Daudin which is most conspucious by its large body size and the thorny warts on its back (Mertens 1947). Although *Bufo bufo* usually occurs low altitudes, it can be found up to an altitude of 2200 metres in the Alps (Angel 1946).

Because of its extended geographical distribution the climatological conditions to which *Bufo bufo* is exposed varies greatly within its geographical area. At northern latitudes winters are usually long and the growing seasons are short, relatively cool and wet (Johannessen 1970). At high altitude in the Alps the weather regime is even more severe (Schüepp and Schirmer 1977). In contrast, at southern latitudes the growing seasons are long, warm and relatively arid (Arléry 1970). It is common knowledge that climatological conditions (temperature and humidity in particular) exert a great influence on many characteristics of amphibian populations (Kühnelt 1970), such as body size distribution, longevity, age structure, body size and age at maturation, growth and so on. This has been confirmed recently by Caetano et al. (1985) concerning the longevity of *Triturus marmoratus marmoratus* from different elevations in Portugal.

In this chapter the results of a skeletochronological study on *Bufo bufo* from different climates and additional field observations are reported and discussed.

MATERIAL AND METHODS

Common toads were captured during their spawning period in five countries of Europe, being the Netherlands, Germany, France, Norway and Switzerland. The five populations will be indicated as NET, GER, FRA, NOR and SWI, referring in this sequence to their country of origin. The toads from NET, GER, NOR and SWI definitely concerned *Bufo bufo bufo.* However, at FRA it was not known whether it was *Bufo bufo bufo,* or *Bufo bufo spinosus* which was studied. The geographic locations of the spawning sites are shown in Fig. 6.1 and described in Table 6.1. Further information is given in chapter 3. The estimated circumferences of the

spawning sites and their surfaces are also given in Table 6.1. The spawning sites of NET and SWI are situated in a rather open area, their banks mainly covered by grassy vegetation. Those of NOR and FRA are situated in a coniferous forest, the spawning site at GER is located in a mixed coniferous and deciduous forest. At FRA the banks are fully overgrown with bushy vegetation such as elder and blackberry, and with willows As a consequence they are mainly shaded The spawning site of GER does not receive much sunlight either, because of the tall vegetation of beech and oak immediately around the fen At NET, NOR and SWI the surface of the water is fully exposed to sunlight All spawning sites are located in a relatively undisturbed area, except for that of NET.

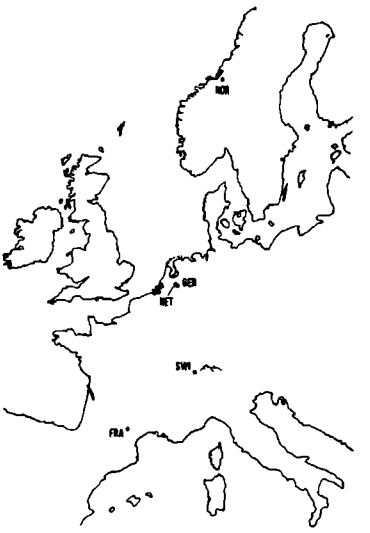

Fig. 6.1 *Geographic location of the spawning sites.*

The populations from NET and GER were studied in 1981, those from the other sites in 1982 Toads from FRA, NOR and SWI were marked individually by toe clipping. In addition the third toe from the left forefoot (denoted ft) was removed from each toad At NET and GER toads were year-marked only by removing ft. The body size of each toad was measured from snout to urostyle to the nearest mm.

Table 6 1. *Geographic locations and sizes of the spawning sites.*

site	latitude	longitude	altitude (m)	circumference (m)	surface (m^2)
NET	51°47'N	5°48'E	15	555	13,500
GER	51°45'N	5°58'E	25	100	500
FRA	43°28'N	2°12'E	500	150	1,000
NOR	63°23'N	10°37'E	150	350	7,500
SWI	46°39'N	8°06'E	1850	85	400

The age of the toads was determined by skeletochronological analysis of the last but one phalanx of ft, according to the method described in chapter 3. The diameter of each year ring present in the phalanx was measured as described in chapter 2 and will be presented as the phalangeal diameter at a particular age If present, the diameter of the bone delineated by the line of metamorphosis was also measured, representing the phalangeal diameter at metamorphosis. By phalangeal growth is meant the increase of the phalangeal diameter.

All toads of both sexes captured at NOR and SWI and all female toads captured at FRA were included in skeletochronological analysis. From the other toads only a sample was selected for this purpose, reflecting the distribution of their body sizes, for each sex and population separately.

At each site, NET excepted, metamorphosing toads were captured. All of them were year-marked by removing a particular toe. The body size of several of these newly metamorphosed toads from GER, NOR and SWI was measured from snout to urostyle (to the nearest 0.1 mm) under a stereomicroscope. Just before hibernation several first-year toads were (re)captured, at FRA, NOR and SWI in 1982 and at GER in 1980 Their body sizes were measured in a similar way as those of the adults.

The statistics used are described by Sokal and Rohlf (1981) and performed by means of SAS (1982), a computer system for data analysis. The level of significance used in the statistics is α=0 05, unless mentioned otherwise When appropiate, α was lowered according to Bonferroni (Dunn 1964)

RESULTS

Activity patterns of the populations

The activity patterns of the five populations are shown in Fig 6.2 and reflect the general situation for the populations concerned. The general activity patterns at NET and GER are known from a long-term study of these populations (from 1975-1982) Information on general activity at FRA, NOR and SWI was gathered from local herpetologists, from the literature and from personal observations in 1982 (and also in 1983 and 1984 for SWI).

At FRA toads emerge from hibernation late in February, a few weeks before those from NET and GER Spawning occurs in two waves at FRA,

the first early in March and the second, being the most largest one, early in April, at about the same time as at GER and NET. At NOR and SWI hibernation lasts until early May and mid-June respectively, eggs being deposited about 2 weeks afterwards. Egg deposition generally occurs within a few days.

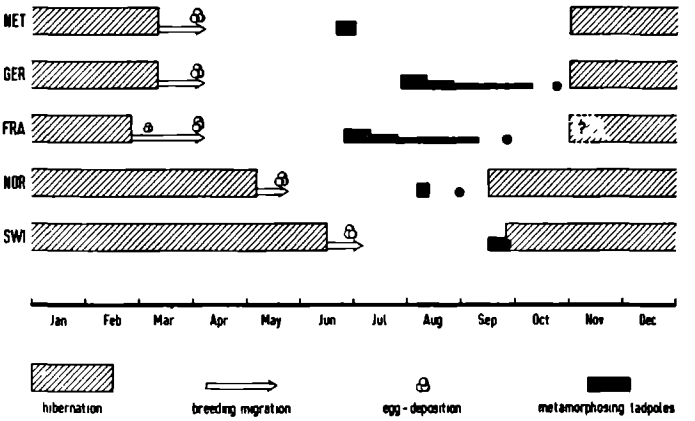

Fig. 6.2. *Activity patterns of the populations.* • = *date of last capture.*

Tadpoles from NET, NOR and SWI metamorphose as little as 10-12 weeks after egg deposition. However, at FRA the first tadpoles metamorphose only 15 weeks after the first eggs are deposited and at GER larval development takes even much longer. Here metamorphosis occurs only 17 weeks after the eggs are deposited. Whereas at NET, NOR and SWI tadpoles metamorphose simultaneously (within 2 weeks), at GER and FRA metamorphosing tadpoles are present during about 11 weeks. Consequently, the time available for growth until hibernation will vary considerably among the newly metamorphosed toads from GER and FRA. As appears from Fig. 6.2, the period from metamorphosis until hibernation varies also among the populations, being the longest at NET and the shortest at SWI.

Assuming that all toads, older than 1 year, start feeding and, thus, may grow only after the spawning period (Jørgensen et al. 1979), the annual growth period of *Bufo bufo* from NET, GER and FRA is of comparable length and about twice as long as at NOR and three times as long as at SWI.

Population size and sex ratio

The number of individual toads captured and the observed overall sex ratio are given in Table 6.2. With capture-recapture data the sizes of the spawning populations were estimated, using the Lincoln-index. The estimated values, also given in Table 6.2, refer to the estimated number of toads in the night that spawning occurred most abundantly, when most of the toads were found to be present at the spawning site. Standard deviations were calculated according to Bailey (1952). The estimated sex ratios were calculated from the estimated number of males and females and are also included in Table 6.2. The size of the female spawning population from FRA was not estimated, because very few females were recaptured. Moreover, the spread egg deposition at this site (cf. Fig. 6.2) suggests an unequal coming and going of female toads, preventing a reliable estimation of their number. Judging from the estimated size of the spawning population and the number of toads actually captured at NOR and SWI, it can be concluded that at those sites almost the entire spawning population had been captured. Both these spawning populations are rather small in comparison with the others

Although the estimated sex ratio is somewhat smaller than that observed, both values indicate that males outnumber females in each population, especially at FRA and NET.

Table 6.2. *Number of individual toads captured (N_c), observed overall sex ratio and estimated population size (N_e) with standard deviation (sd) and estimated sex ratio.*

site	N_c	sex ratio observed	N_e	sd	sex ratio estimated
NET ♂♂	1178	5.75	9539	1115	5.66
♀♀	205		1684	621	
GER ♂♂	777	1.99	2437	203	2.02
♀♀	391		1206	381	
FRA ♂♂	1545	16.26	6803	897	
♀♀	95				
NOR ♂♂	208	2.48	209	6	2.06
♀♀	84		101	13	
SWI ♂♂	82	2.41	92	14	1.92
♀♀	34		48	13	

Body size of first-year toads

In Table 6.3 the average body sizes of metamorphosing toads from GER, NOR and SWI are given. These body sizes differ markedly from each other (*t*-tests, α=0.01, all three P<0.0001), the smallest being measured at GER, the largest at SWI. As the tadpoles from SWI metamorphosed only in September, just before hibernation starts (see Fig. 6.2), it is supposed that they did not grow any further. From Table 6.3 it appears that, on the average, the first-year toads from the other sites did increase their body sizes towards hibernation, although at GER and FRA there was still a number of very small toads. The average body sizes at the last capture differ significantly among the populations (Wilcoxon sign-rank tests, α=0.01,

Table 6.3. *Average body size of first-year toads at metamorphosis and at last capture, before hibernation.*
n = number, av = average body size, sd = standard deviation, w-exp = minimum and maximum number of weeks experienced after metamorphosis, w-left = weeks left before hibernation.

	METAMORPHOSIS				BEFORE HIBERNATION					
site	n	av	sd	range	n	av	sd	range	w-exp	w-left
GER	12	7.9	.38	7.2- 8.6	26	17.6	4.0	8-25	2-13	1
FRA					232	15.2	2.9	9-25	2-13	≥5
NOR	12	9.7	.56	8.8-10.4	470			12-18	3- 4	1-2
SWI	15	12.5	.62	11.5-13.5	44	12.1	0.7	11-13	1	0

all P<0.001, NOR not included), the largest being those from GER, the smallest those from SWI. However, the first-year toads from FRA are expected to grow for about another five weeks before hibernation (see Table 6.3 and Fig. 6.2), probably then reaching an average body size closer to that of first-year toads from GER. The average body size at the last capture at NOR is not given, as the body sizes of only the smallest and largest toads out of 470 toads captured were measured. Nevertheless, at hibernation they will be smaller on the average than those from FRA and GER and larger than those from SWI.

Body size and catchability of the adult toads

The body size distributions of the adult toads captured at each site are represented in Fig. 6.3. In each spawning population female toads are larger than male toads (*t*-tests, all $P<0.0001$). The average difference between both sexes amounts to about 12 mm, except at FRA where females are on the average 18 mm larger than males. Among the five spawning populations body sizes differ markedly for both sexes (one-way analysis of variance, both $P<0.0001$). Pairwise comparisons with *t*-tests at $\alpha=0.005$, showed that in each population the body sizes differ from those in all other

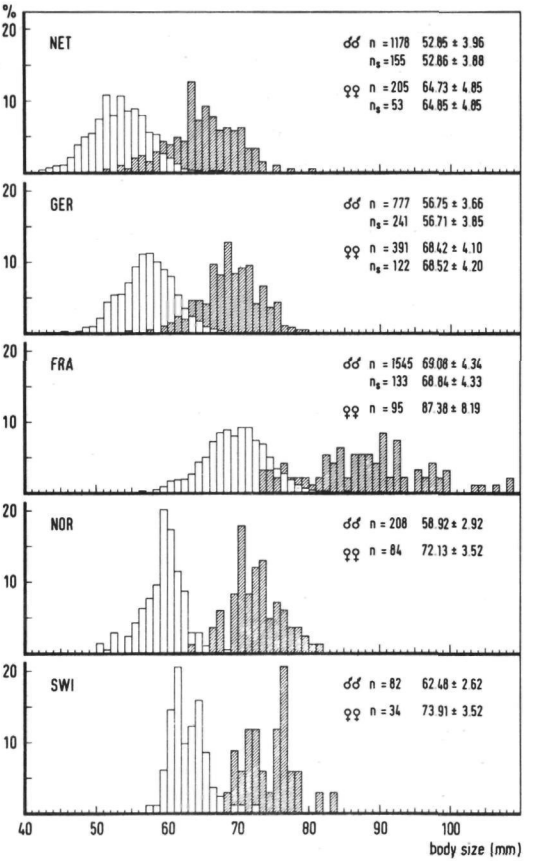

Fig. 6.3. *Body size distribution of the individual toads captured in each spawning population. Sample size, average body size and standard deviation of the total sample and, where relevant, those of the sample selected for skeletochronological analysis (n_s) are also included.* ♂♂ = □, ♀♀ = ▨.

populations (males all $P<0.0001$, females NOR-SWI $0.01<P<0.02$, other $P<0.0001$). The smallest males and females were measured at NET (42 mm and 51 mm, respectively), the largest at FRA (85 mm and 108 mm, respectively).

Many toads, males in particular, were recaptured at least once. At NOR and SWI this applied to 88% and 41% of the male toads and 60% and 56% of the female toads. Over 50% of these toads were recaptured more than once (up to 7 times). If each individual had an equal chance of being captured, the ratio of small to large toads in the recaptures should be the same as in the original captures. Table 6.4 shows the observed and the expected number of recaptures with body sizes smaller and larger than the average body size in the population concerned. Although in general large toads are more frequently recaptured than expected, this appears to be significant only for male toads from NET and for female toads from GER (binomial tests, P-value indicated in Table 6.4).

Table 6.4. *Observed number of recaptures (O), being smaller (< av) and larger (> av) than the average body size of toads from the spawning population concerned, and expected numbers (E) when equal catchability was supposed. Binomial tests are used for statistics.*

	NET		GER		FRA		NOR		SWI	
	O	E	O	E	O	E	O	E	O	E
MALES										
< av	78	94	150	163	71	73	337	337	24	32
> av	128	112	199	186	70	68	273	273	35	27
statistic	p<.05		p>.20		p>.70		p=1		.05<p<.1	
FEMALES										
< av	8	6	14	22	2	4	37	43	18	24
> av	5	7	28	20	6	4	58	52	24	21
statistic	p>.40		p<.04		p>.20		p>.40		p>.60	

As was mentioned above, a sample was selected from the toads captured at NET and GER and from the male toads captured at FRA. The selected samples reflect the body size distribution of the toads captured at the spawning site concerned (see Fig. 6.3).

Age structure of the spawning populations

Fig. 6.4 shows the age structure of the spawning populations. Toads the age of which might be underestimated by more than one year were not included in the Figure. This applied to relatively many females from NOR, FRA and NET and to males from NOR (cf. n_s from Fig. 6.3). Toads which are included in the Figure could not always be assigned to one particular age class either (blocks overlapping 2 years). In further analyses only toads of known age will be considered.

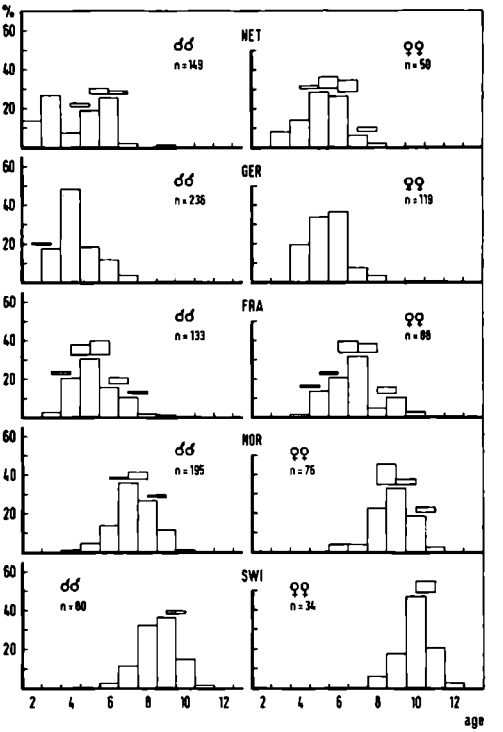

Fig. 6.4. *Age structure of the spawning populations. Blocks overlapping two age classes refer to toads that could not be placed in any of these age classes with certainty.*

In Fig. 6.4 the populations are arranged in order of increasing age of the youngest adults. The youngest males and females were found at NET, being 2 and 3 years old, respectively. The youngest males and females from SWI were 6 and 8 years old, respectively.

The age structure of male toads in the spawning population from NET is rather irregular Age class 6 is very numerous compared to the other age classes, whereas on the contrary relatively few toads of 4 years old are present at the spawning site. The latter also applies to the number of toads in the youngest age class, not only in the age structure of the spawning population from NET, but also in that of the other spawning populations In fact, in most spawning populations the most numerous age class occurs somewhere in the middle of the age distribution, most likely indicating that relatively few individuals of the younger age classes have migrated to the spawning site.

In each spawning population the youngest females are at least one year older than the youngest males, and they also continue spawning to a more advanced age As expected from this, female toads at the spawning site are older on the average than male toads on the average (Wilcoxon sign-rank tests, all $P<0.0001$). Among the spawning populations the age structures differ markedly as apparent from Kruskal-Wallis one-way analysis of variance (for each sex separately, both $P<0.0001$) Pairwise Wilcoxon sign-rank tests at $\alpha=0.005$ showed that only the age structures of the spawning populations from NET and GER do not differ ($P>0.27$ for both sexes) The age structure of the other spawning populations differ from each other and also from that of NET and GER ($P<0.0001$)

Body growth and phalangeal growth

For each age class the average body size and standard deviation of adult toads are shown in Fig 6 5. To investigate the effect of sex and age on adult body size, two-way analyses of variance were carried out for each population separately They showed a significant effect of sex on adult body size (all $P<0.0001$). Further investigation of the sex effect was performed with *t*-tests at each comparable age. Sexual dimorphism concerning body size was found at all comparable adult ages ($\alpha=0.01$, all $P<0.0015$) A significant effect of age on adult body size was only found in the populations from NET, GER and NOR (these $P<0.0006$), indicating that in these populations body size generally increases with age. The age effect on adult body size in the three populations concerned was further investigated using *t*-tests at $\alpha=0.01$ between the adjacent ages, for each sex separately. Significant differences were found incidentally and only between the younger age classes.

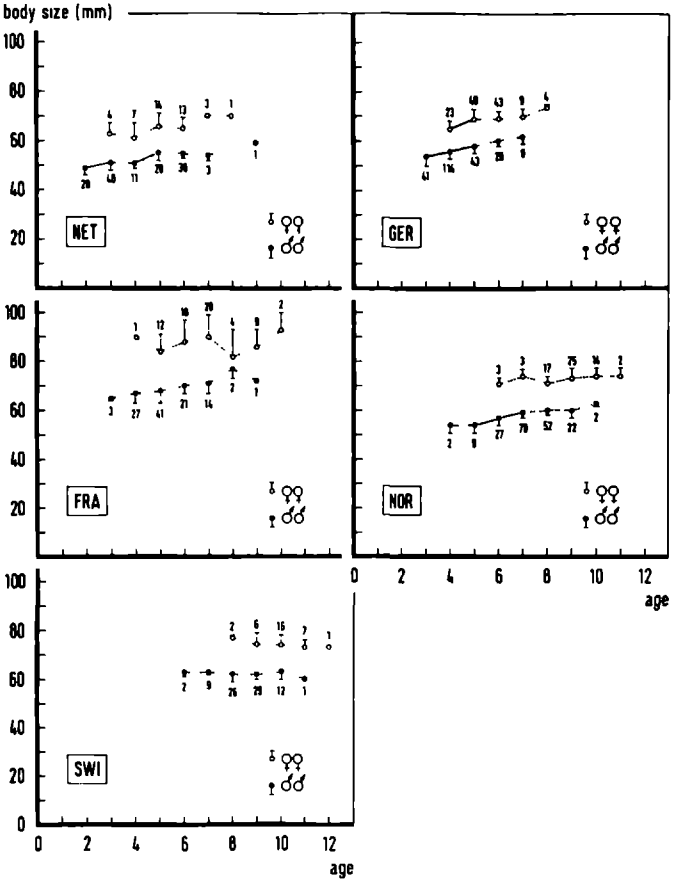

Fig. 6.5. *Average body size and standard deviation of adult toads in each age class. Number of toads are indicated. Solid lines indicate significant differences between the adjacent age classes.*

The diameter of a year ring represents the phalangeal diameter at the end of a growing season as well as at the start of the next one after hibernation and spawning (if any). In Fig. 6.6 the average phalangeal diameter and standard deviation of the successive year rings in the phalanges of the toads studied are given, thus giving information about the phalangeal growth during the successive growing seasons. As *Bufo bufo* experiences only one growing season a year, Fig. 6.6 actually reflects the annual phalangeal growth in toads. In the Figure phalangeal growth after metamorphosis is given, the age at metamorphosis being expressed as the

proportion of the growing season already lapsed at that time. Obviously, the age at metamorphosis expressed in this way varies among the populations (cf. Fig. 6.2).

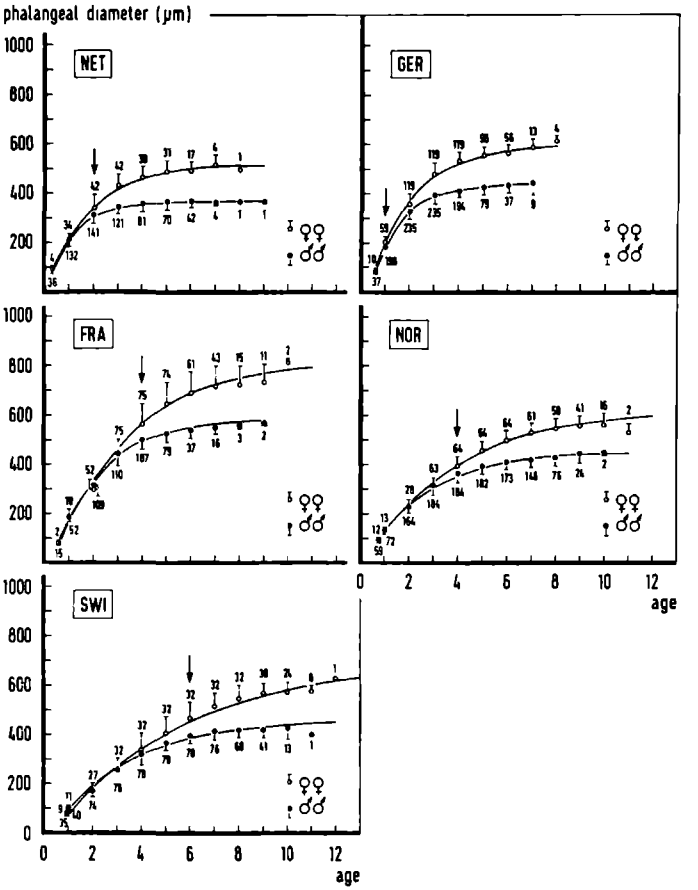

Fig. 6.6 *Average phalangeal diameter and standard deviation at different ages. Time interval between adjacent ages is the annual growing season. Number of toads are included. Arrows indicate the age at which sexual dimorphism is significant. Von Bertalanffy growth curves are included.*

The average seasonal body growth and phalangeal growth of a cohort of toads after metamorphosis can be described by a negative exponential function of time. The general form of this equation, the von Bertalanffy

growth equation (1938), is:

1) $S_t=S_m-(S_m-S_0)e^{-k(t-t_0)}$ residual standard deviation σ

in which

- t = number of growing seasons experienced, that is age
- t_0 = proportion of the growing season already lapsed at metamorphosis, that is age at metamorphosis
- S_t = average body size or phalangeal diameter after having experienced t growing seasons, that is at age t
- S_m = average maximal body size or phalangeal diameter
- S_0 = average body size or phalangeal diameter at metamorphosis, that is at age t_0
- k = growth coefficient, defining the shape of the growth curve. A high value of k indicates that S_m will be achieved soon.

This growth model implies that the growth *rate* of a cohort is a function of body size or phalangeal diameter, and invariable with time. An approximation of it may be given by the derivation of the von Bertalanffy growth equation, being a (decreasing) linear function of S:

2) $\text{growth rate} = dS/dt = k(S_m-S_t)$

Growth rate is maximal when S_t is minimal, which means at metamorphosis when $S_t=S_0$.

As no sufficient data on body size of juvenile toads and young adults were available, only the growth equations of phalangeal growth were computed. The NLIN procedure (option MARQUARDT) of SAS (1982), providing an iterative procedure for asymptotic least-squares estimates of non-linear parameters, was used to fit the growth model to the data, for males and females and for each population separately. The age at metamorphosis (t_0) might be estimated from field observations (see Fig. 6.2). However, as metamorphosis occurs during several weeks (even up to 11 weeks at FRA and GER), it is difficult to estimate the average age at metamorphosis from these observations, especially since the number of metamorphosing toads per week was not recorded. Therefore, it was preferred to vary t_0 for both sexes and for each population between the extremes as estimated from Fig. 6.2. With each value adjusted to t_0 the phalangeal growth equation was computed. Taken into consideration that t_0 in a particular population should be equal for males and females, that value adjusted to t_0 at which the

residual standard variation of the growth equation was as small as possible, was chosen to represent the average age at metamorphosis in the population concerned

Table 6.5 *Von Bertalanffy equations for phalangeal growth and the residual standard deviations (σ). The asymptotic standard errors and the 95% asymptotic confidence limits of the estimated parameters are also included.*

site	$S_t=S_m-(S_m-S_0)e^{-k(t-t_0)}$	σ	standard errors			95% confidence limits		
			S_m	S_0	k	S_m	S_0	k
MALES								
NET	$368-(368-75)e^{-.92(t-.32)}$	28	2.3	4.4	.030	364-373	67-84	.86-.98
GER	$433-(433-82)e^{-.86(t-.60)}$	30	2.4	3.6	.023	429-438	75-88	.82-.91
FRA	$589-(589-84)e^{-.47(t-.54)}$	43	7.9	7.4	.021	574-605	70-99	.43-.51
NOR	$449-(449-88)e^{-.42(t-.75)}$	31	2.9	2.9	.011	444-455	82-94	.40-.44
SWI	$462-(462-81)e^{-.32(t-.95)}$	35	5.8	3.8	.013	451-474	73-88	.30-.35
FEMALES								
NET	$520-(520-70)e^{-.56(t-.32)}$	45	10.2	14.9	.045	500-540	41-100	.47-.65
GER	$597-(597-88)e^{-.58(t-.60)}$	39	5.1	7.1	.020	587-607	74-101	.54-.62
FRA	$826-(826-22)e^{-.32(t-.54)}$	77	20.2	23.4	.028	787-866	-24-68	.26-.36
NOR	$623-(623-82)e^{-.27(t-.75)}$	39	9.4	7.5	.013	604-641	67-97	.24-.29
SWI	$708-(708-67)e^{-.18(t-.95)}$	55	27.6	10.3	.015	653-762	47-88	.15-.21

The phalangeal growth curves are represented in Fig. 6.6 and their equations in Table 6.5. The asymptotic standard errors of the estimated parameters and the residual standard deviations (Table 6.5) indicate that the model fits quite well, but better for males than for females The estimated S_0 resembles the average value of this parameter measured in the adults, except for the females from FRA and SWI. The average maximal phalangeal diameter (S_m), however, does not represent one particular value from the distribution of the phalangeal diameters in adult toads. In male and female toads from NET and GER and in males from NOR, S_m equals the average phalangeal diameter of the toads from the older age classes. In males from FRA and SWI, S_m is somewhat larger than this value and in females from FRA and NOR, S_m represents the largest value measured in

those adults. In females from SWI, S_m is larger than the largest value measured. Taken all together, the estimated parameters for male toads are more accurate (less bias) and more precise (lower asymptotic standard errors) than those for female toads.

Although the phalangeal diameter at metamorphosis (S_0) does not differ among toads -evidently not in agreement with the differences found among the body sizes of metamorphosing toads from GER, NOR and SWI (cf. Table 6.3)- , the maximal phalangeal diameter does, both between the sexes and among the populations. The differences in the maximal phalangeal diameters reflect the sexual dimorphism and the interpopulational differences in body size found before (cf. Fig. 6.3). This is not surprising, as the phalangeal diameter was found to be positively related to body size in adults (Pearson r ranging from 0.36 to 0.64 in males and from 0.62 to 0.72 in females). Although some of the correlations are relatively weak, they all appear to be significant ($P<0.003$).

The growth coefficient k also differs markedly between the sexes and among the populations, indicating that phalangeal growth proceeds differently in males and females from each population. Yet the growth rate at metamorphosis (R_{init}), calculated with equation 2 and $S_t=S_0$, corresponds rather well in males and females (Table 6.6), suggesting that males and females initially grow at the same rate. The sexual dimorphism concerning phalangeal growth becomes significant from the age of the youngest adult males in each spawning population or one year before (FRA excepted), as can be seen in Fig. 6.6 (the arrows indicate the age from which the phalangeal diameter in female toads is significantly larger than in male toads, t-tests at $\alpha=0.005$). The larger female body size at each comparable

Table 6.6. *Phalangeal growth rates at metamorphosis [$R_{init}=k(S_m-S_0)$] and at the minimum age of maturation [$R_{mat}=k(S_m-S_{mat})$] in µm/growing season. R_{mat} relative to R_{init} is also included. Further explanation see text.*

	MALES			FEMALES		
	R_{init}	R_{mat}	R_{mat}/R_{init}	R_{init}	R_{mat}	R_{mat}/R_{init}
NET	270	52	0.20	252	56	0.22
GER	302	63	0.21	295	62	0.21
FRA	237	58	0.24	257	67	0.26
NOR	152	32	0.21	146	38	0.26
SWI	122	25	0.21	115	33	0.28

adult age is consistent with this (cf. Fig. 6.5). As appears from Table 6.6, among the populations the growth rates at metamorphosis do vary, being the smallest at SWI and NOR and the largest at NET and GER.

The growth coefficient k also seems to reflect the relative differences that were found with respect to the age of maturation, both between the sexes and among the populations. A high value of k is associated with an early age of maturation (cf. Fig. 6.4). The average minimum number of growing seasons experienced at maturation can be calculated by considering the relative growth equation and the frequency distribution of the phalangeal diameters of the adults when captured (for each sex and population separately). The value of the phalangeal diameter beyond which 95% of the diameters were measured (S_{mat}) was considered the minimum phalangeal diameter to be reached before the individual can attain sexual maturity. S_{mat} and the average number of growing seasons to be experienced before S_{mat} is reached, that is the average minimum age of maturation, are given in Table 6.7. The Table shows that S_{mat} varies between the sexes and among the populations in the same way as the maximal phalangeal diameter does, being the lowest at NET and the largest at FRA. The theoretical calculations of the average minimum age of maturity agree quite well with the age of the youngest adults captured in the spawning populations (cf. Fig. 6.4), except for GER.

Table 6.7. *Average minimum phalangeal diameter (S_{mat}, µm) and average minimum age (A_{mat}, number of growing seasons experienced) at maturation, as calculated with the phalangeal growth equations.*

site	MALES		FEMALES	
	S_{mat}	A_{mat}	S_{mat}	A_{mat}
NET	311	2.1	420	3.0
GER	360	2.4	490	3.3
FRA	466	3.6	617	4.8
NOR	372	4.4	527	8.0
SWI	384	5.9	527	8.0

The growth rate at the minimum age of maturation (R_{mat}), calculated with equation 2 and $S_t=S_{mat}$, is included in Table 6.6. As can be seen in this Table, R_{mat} is reduced to about 21% of the initial growth rates in males (FRA not considered). Concerning the females this percentage varies from

about 21% at NET and GER to 26%-28% at the other sites.

The maximal percentage of sexually mature toads in each age class was estimated with (S_{mat}) and the average phalangeal diameter and its standard deviation at that age, according to the way described in chapter 5 (Discussion). In Table 6.8 the estimated percentages are given according to sex and population. The variation in the age at which an individual may attain maturity is the largest in populations with a short annual growing season (NOR and SWI). Although the estimated percentages generally reflect the age structure found in the spawning population, some differences can be noticed. According to Table 6.8, toads from GER and SWI and female toads from NOR may reach sexual maturity at an earlier age than actually was found among the toads in these spawning populations.

Table 6.8. *Average maximal percentage of toads in each age class that can be sexually mature. Further explanation see text and chapter 5 (Discussion).*

		AGE CLASSES											
site	sex	1	2	3	4	5	6	7	8	9	10	11	12
NET	♂♂		35	82	>95								
	♀♀			50	83	94	>95						
GER	♂♂		14	83	>95								
	♀♀			30	82	>95							
FRA	♂♂			22	71	92	>95						
	♀♀				23	58	82	92	>95				
NOR	♂♂				30	70	88	>95					
	♀♀					20	58	84	95				
SWI	♂♂					20	50	74	85	91	95		
	♀♀						08	26	51	71	84	92	>95

As was mentioned before, the growth coefficient k indicates when the maximal phalangeal diameter S_m is reached. The age at which the phalanx reaches 95% of its maximal diameter will be considered here as the age at which the phalanx is full-grown. These ages are shown in Table 6.9. Apparently, female toads continue phalangeal growth much longer than male toads. As a matter of fact, females from SWI and NOR do not live long enough to reach 95% of S_m according to the growth equation. The age at which the phalanx was calculated to be full-grown corresponds to the age at

which 95% of the toads were calculated to have attained maturity (cf. Table 6 8), except for the females from FRA, NOR and SWI.

Table 6.9. *Age (number of growing seasons lapsed) at which 95% of the maximal phalangeal diameter (S_m) is reached, as calculated with the phalangeal growth equations.*

	NET	GER	FRA	NOR	SWI
males	3.3	3.8	6.6	7.4	9.7
females	5.4	5.5	9.8	11.3	17.0

Up to now, parameter t in the equations has been expressed as the number of growing seasons lapsed (= age) However, when comparing growth and growth rates among the populations, it must be realized that the length of the growing season and the proportion of the growing season lapsed at metamorphosis (t_0) vary among the populations. Therefore, the growth equations were recomputed, expressing t as the actual time (in years) spent on growth. This time will further be indicated as the number of growth years. It is evident that only the value of k and t_0 will change as a result of this new calculation, to be divided by and to be multiplied with the proportion of a year available for growth (=G), respectively. The recomputed growth curves for phalangeal growth after metamorphosis are shown in Fig 6.7, and the actual growth coefficient k and its 95%

Table 6.10. *The actual growth coefficient k when time is expressed in growth years (cf. Table 6.5), its standard error (s.e.) and the 95% asymptotic confidence limits (c.l.). The proportion of the year available for growth (G) and the actual age at metamorphosis (t_0) are also included. Further explanation see text.*

			MALES			FEMALES		
site	G	t_0	k	s.e.	95% c.l.	k	s.e.	95% c.l.
NET	588	.19	1.56	.050	1.46-1 66	.95	.077	.80-1.11
GER	588	.35	1.47	.039	1.39-1.54	.98	.034	.92-1.05
FRA	588	.32	.80	.036	.73- .87	.54	.042	.45- .62
NOR	.292	.22	1.45	.036	1.38-1.52	.91	.042	.83- .99
SWI	218	.21	1.44	.058	1.33-1.55	.84	.075	.69- .99

asymptotic confidence limits (c l.) are given in Table 6.10. The actual age at metamorphosis and the estimated proportion of a year available for growth (G) are also included in this Table Of course the differences between both sexes in each population with respect to phalangeal growth remain unchanged. However, the interpopulational differences in the growth coefficient exist no longer Although the value of the actual growth coefficient of females from SWI and NOR is somewhat smaller than that of the other females, all actual growth coefficients are equal within their 95% c l for toads of the same sex, those from FRA excepted. The average actual k for male and female toads amounts to 1.48 and 0.92, respectively (toads from FRA not included) As the actual growth coefficient k is equal for toads of the same sex and S_m is not, it is evident that the growth rates per growth year vary among the populations (see equation 2). At equal S_t the growth rate per growth year of toads from SWI is the largest, that of toads from NET the smallest (FRA not considered)

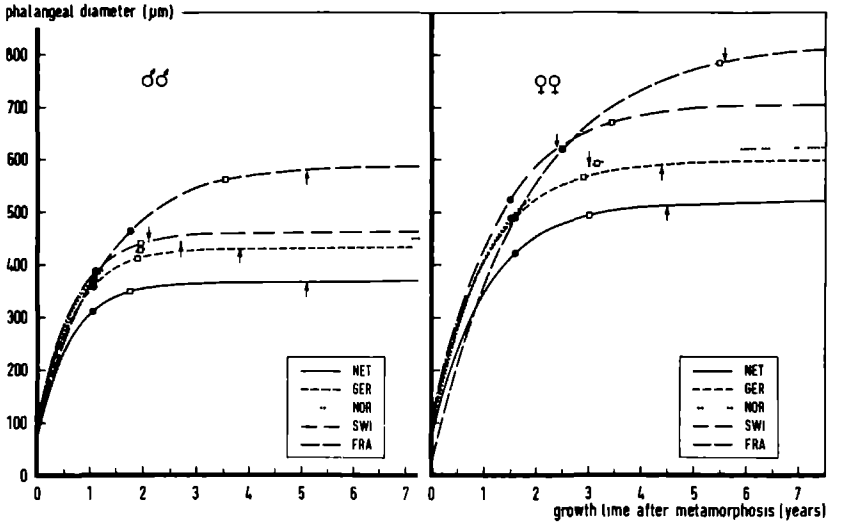

Fig. 6.7. *Von Bertalanffy growth curves for phalangeal growth, when time is expressed in growth years. • = average minimum number of growth years lapsed at maturation; □ = average number of growth years lapsed at which 95% of the maximal phalangeal diameter is reached; ↑ or ↓ = number of growth years experienced by the oldest toads. For further explanation see text.*

The equal k means that phalangeal growth proceeds equally Thus, the phalanx of toads of the same sex is full-grown after an equal number of growth years. As can be seen in Fig. 6.7 (open squares), the number of growth years lapsed when the phalanx reaches 95% of its maximal diameter ranges from 1.77 to 1.95 (averaging 1.88) for male toads, males from FRA not included. For female toads the range is larger, from 2.90 to 3.45 growth years (averaging 3 11), females from FRA not included.

From Fig. 6.7 it also appears that the oldest toads in the different populations had not experienced an equal number of growth years (the arrows indicate the number of growth years experienced by the oldest toads in each population). The oldest toads from SWI and NOR, although considerably older, had lapsed less growth years than those from GER and NET. Apparently, their older age does not compensate entirely for the shorter growing seasons.

The minimum number of growth years lapsed before maturity can be reached is indicated in Fig. 6.7 as closed circles. The minimum number of growth years lapsed at maturation averages 1.09 for males (ranging from 1 07 to 1 15) and 1.55 (ranging from 1.50 to 1.59) for females, toads from FRA not included.

DISCUSSION

Catchability

From the fact that there was a tendency for large toads to be recaptured more frequently than small toads, it may be concluded that large toads have a higher chance of being captured than small toads A similar unequal catchability was reported before in chapter 5 and also by van Gelder and Rijsdijk (manuscript in prep), both for *Bufo bufo* from GER. It can also be concluded from the studies of Clarke (1972) on *Bufo woodhousei fowleri* and of Gatz (1981) on *Bufo americanus*. When all toads of a spawning population have been captured, as at NOR and SWI, unequal catchability evidently is of no importance, because it merely implies that large toads are simply *re*captured more frequently than small toads. However, if only a part of the spawning population has been sampled, it would imply that relatively few small toads have been captured. If the catchability of a toad of a given body size is known, the sample can easily be corrected for unequal catchability. If not, unequal catchability could

affect the validity of the sample as being representative for the entire spawning population, at least with respect to the body size frequency distribution and to other population characteristics related to body size. This could apply to the spawning populations from NET, GER and FRA. However, unequal catchability is not likely to be responsible for the sexual and interpopulational differences found with respect to the parameters studied.

Larval development

Bizer (1978), Berven et al. (1979) and Berven (1982*b*) found that in cold environments larval development of *Ambystoma tigrinum, Rana clamitans* and *Rana sylvatica* takes longer and metamorphosis occurs at larger body size than in warm environments. The replacement experiments of Berven (1982*b*) with lowland and mountain larvae of *Rana sylvatica* clearly showed that temperature, although the most important, was not the only factor which determines larval body size and larval development rate. Part of the variation could be ascribed to genetic differences in growth rates and to non-genetic maternal effects on development rates, which resulted in a relatively *faster* larval development and in larger body sizes at metamorphosis of mountain larvae. To which degree this also applies to *Bufo bufo* is unknown; the results do not allow to draw definite conclusions. However, in general they are not contradictory to those of Berven (1982*b*), except for the unexpectedly long period of larval development at FRA and GER. As also here the period of metamorphosis is extended, it is assumed that another (additional) factor is responsible for the long larval development at FRA and GER and for its greater variability.

Validity and reliability of the growth model

Average phalangeal growth of cohorts after metamorphosis was described by the von Bertalanffy (1938) growth equation for linear growth, despite the criticisms of Cloern and Nichols (1978) and those of Roff (1980) against its use. Cloern and Nichols (1978) rightly said that the function is inappropriate for organisms the growth of which is restricted to a seasonal period. Indeed, by using a constant k as the growth coefficient, it is assumed that the growth rate is a constant function of size alone and invariable with time. Concerning *Bufo bufo* this assumption is not valid, because seasonal variations in the growth rate exist, even when only the

growing seasons are considered. Most likely, these variations are related to seasonal variations in environmental temperatures and/or in abundance and quality of food during the growing season. In fact, Heusser (1968*b*) found that toads do not feed until the temperature has risen up to about 11-12°C, and Jørgensen et al. (1979) mentioned that toads probably do not spend much time on feeding during the month following spawning. By introducing a time-varying growth coefficient, Cloern and Nichols (1978) made the von Bertalanffy growth equation more appropriate for the description of such variable growth. Unfortunately, it was not possible to estimate a time-varying coefficient for the growth of *Bufo bufo* in this study, because data about size were only known at one fixed moment each year, after hibernation. Thus, no data about sizes during the growing season were available. Therefore, the von Bertalanffy growth equation with the constant growth coefficient k does not describe correctly the average intraseasonal growth of individuals of a cohort. Yet it is a simplified but reasonable approximation of it. This simplification implies, that wherever in the results predicted values are given, which will be reached in the course of the growing season, these values might not be quite accurate However, possible inaccuracies certainly will not be so large that they can invalidate the conclusions about sexual and interpopulational differences or similarities.

Roff (1980) stated that one of the major disadvantages of the von Bertalanffy growth equation is the extreme difficulty of fitting the model to the data in a statistically satisfactory manner However, Bayley (1977) already proposed a linear solution to the equation in which the von Bertalanffy parameters could be estimated with confidence limits. In this study the equations were fitted by the iterative non-linear procedure (NLIN) of SAS (1982), which was recommended by Vaughan and Kanciruk (1982). NLIN can be easily performed and also yields asymptotic standard errors for each parameter estimated, so that they can be compared with those of other equations. Moreover, Vaughan and Kanciruk (1982) convincingly showed that NLIN of SAS (1982) is superior to two linear solutions, including that of Bayley (1977). When estimated according to NLIN, the parameters were found most accurate (least bias) and most precise (lowest variability). Furthermore, Vaughan and Kanciruk (1982) found that NLIN is least sensitive to changes in the underlying conditions, such as the total number of observations, time interval between the observations and variability of the data. So the main arguments of Roff (1980) against the use of the von Bertalanffy growth equation are taken

away, when fitting is performed with NLIN of SAS (1982).

The reliability of the growth equations is supported by a study on the toads from SWI in 1984 (unpublished data): the same toe (ft) was removed from the toads then captured, from either the left or the right forefoot. The equations for phalangeal growth, estimated with these data, resemble closely those for SWI given in this chapter and all parameters are equal within their 95% asymptotic confidence limits. However, reliable estimates of the parameters may be hampered for several reasons. According to Knight (1968), parameters will be estimated to nonsense values when there is no (sufficient) curvature in the growth curve. This might be the case when data are available for only the youngest age classes (the steep part of the curve), or for only the oldest age classes (asymptotic part of the curve). In the former case, Vaughan and Kanciruk (1982) found that even then NLIN provides acceptable estimates of the parameters. The latter occurs for instance, when the body sizes of the adult toads would be fitted to the growth model. Indeed, the lack of (sufficient) data about the body sizes of toads from the younger age classes (especially at NOR and SWI) was the major reason for not estimating the equations for body growth. It is probably for the same reason that Gibbons and McCarthy (1984), when estimating the von Bertalanffy equation for body growth of adult *Rana temporaria*, found such a large variability, that they unexpectedly had to conclude that the male and female growth equations were not significantly different. There is another reason for omitting the equations for body growth and preferring those for phalangeal growth. The body size of toads of a particular cohort is the ultimate result of their growth during the preceding years. Since the environmental circumstances vary from year to year, each cohort will have a different growth history Consequently, the average body size of toads of different cohorts might differ considerably when compared at equal ages. This was already demonstrated in chapter 5. Therefore, fitting the growth model to data on body sizes, sampled in only one year might yield less reliable results. With respect to phalangeal diameters this difficulty has not been encountered. With just one single sample, phalangeal diameters of toads of different cohorts at each preceding age are available, the average phalangeal diameter at a given age thereby representing an average value over several years.

Although Vaughan and Kanciruk (1982) stated that the age class sample sizes need not to be equal, the model was found to be sensitive to the number of observations on the phalangeal diameter at metamorphosis (S_0). The results show that when only few S_0 are known (like in most females),

both the precision of the estimated S_0 (large asymptotic standard error) and its accuracy (large bias) are poor. Even an impossible value of S_0 could be estimated, as was found for females from FRA. However, when the phalangeal diameters at metamorphosis of the male toads from FRA where added to those of the females, the estimated S_0 is almost tripled and its asymptotic standard error is reduced with 26%, S_m and k remaining relatively unaffected. The relatively poor accuracy and the large variability of S_0 is probably the reason why the phalangeal diameter at metamorphosis, being equal among the populations, does not reflect the interpopulational differences concerning body size at metamorphosis. On the other hand, it must be kept in mind that the body sizes at metamorphosis, found in this study, represent only the observations from one particular year, which might be not representative for the average body size of metamorphosing toads in the population concerned.

The specific growth coefficient k

The activity patterns show that at northern latitude and high altitude the hibernation period of the toads is prolonged. Consequently their spawning period is delayed and their growing season is shortened compared to that of toads from southern latitude and low altitude. Similar results were found by Holms (1982) and Beattie (1985) for *Rana temporaria* from different altitudes in England. It is commonly known that during hibernation all processes of life are retarded: respiration is lower and heart-beat is reduced in frequency, metabolism is reduced to a minimum (Smith 1964) and growth ceases. From this, it is not surprising that phalangeal growth proceeds differently among populations in which the length of the hibernation period and, thus, that of the growing season varies. The different phalangeal growth, being reflected by k, shows that it takes more years to achieve the maximal dimension S_m in populations with a short growing season than in those with a long growing season. However, when the actual time spent on growth (growth years) is considered, the interpopulational differences concerning k exist no longer (FRA excluded), and only the sexual dimorphism remains. The actual k averages 1.48 for male and 0.92 for female toads. From the fact that in chapter 5 an equal k was found for the growth of both the body and the phalanx ft, it is plausible to assume that k describes all linear growth processes that are sexually dimorphic. Hence, k might be considered to represent a (sexually dimorphic) specific growth coefficient.

Once knowing the specific growth coefficient k, it is possible to formulate the equation for the average body growth of cohorts after metamorphosis, without having data available on body sizes at different ages. The parameters to be estimated are the body size parameters S_m and S_0, and the parameters t_0 and G. G is required when conversion is wanted from the number of growth years to the number of growing seasons lapsed after metamorphosis (=age), and from the actual age at metamorphosis to that expressed as proportion of the annual growth period. The actual age at metamorphosis (t_0) and the annual growth period (G) can be estimated directly from observations on the population concerned. In analogy to S_0 in the phalangeal growth equation, S_0 in the body growth equation represents the average body size at metamorphosis. It can be estimated from the body size distribution of the metamorphosing toads. The average maximal body size S_m cannot be estimated directly from the body size distribution of the adults, as it is not clear which value should be considered as S_m (cf. S_m in the phalangeal growth equations). The average maximal body size S_m can be estimated via the average minimum body size at maturation, which, in analogy to S_{mat} of the phalanx, can be considered the body size beyond which 95% of the body sizes of the adults were measured. With the average minimum body size at maturation and the number of growth years required to reach it (1.09 for males and 1.55 for females), the average maximal body size S_m can then be calculated from the equation. However, as was mentioned before, it must be realized that both the body size distribution and the activity patterns may vary from year to year. Therefore, these parameters should be preferably estimated from observations covering several years.

Average minimum age and size at maturation

Apparently, selection favours a delayed maturation in female *Bufo bufo*, implying that the profit by delaying maturity is greater than the loss suffered by decreased survival. One of the profits of delaying maturation is the greater fecundity at larger female body size, as reported in many herpetological studies (e.g. Rose 1966, Bruce 1969, Kozlowska 1971, Davies and Halliday 1977, Kadel 1977, Semlitsch 1980, Tilley 1980, Berven 1982*a*), although Gittins et al. (1984) found only a poor relationship between female fecundity and body size. In males, the lower reproductive costs (Bruce 1975, Jørgensen 1982) and the questionable relationship between mating success and size (cf. for Bufonidae: Davies and Halliday 1979, Wells 1979,

Gatz 1981, Woodward 1982, Reading and Clarke 1983, Lamb 1984 versus Licht 1976, Wilbur et al. 1978, Kruse 1981, Sullivan 1982 and 1983) may result in a selection towards early maturation, as suggested amongst others by Bell (1977). Such selection, acting in different directions for both sexes, may lead to the observed sexual dimorphism concerning the minimum age and size at maturation in each population of *Bufo bufo* studied.

The interpopulational differences concerning the average minimum age and size at maturation satisfy the expectation that toads from populations with a short growing season mature later and at larger sizes than those from populations with a long growing season. The same was found by Pettus and Angleton (1967), Tilley (1973), Licht (1975) and Berven (1982*a*) for amphibian populations from high and low altitude. However, when only the actual time spent on growth was considered, sexual maturity may be reached after the same number of growth years in each population (FRA disregarded), averaging 1.09 and 1.55 years for males and females respectively. Apparently, attaining sexual maturity in *Bufo bufo* is dependent on a fixed minimum number of growth years, suggesting that this minimum is a constant for *Bufo bufo*. As the environment determines how much time is available for growth each year, the minimum number of growing seasons to be lapsed before maturation is variable among the populations. The average minimum number of growing seasons to be lapsed before maturation (= average minimum age of maturation) can thus be estimated in any population, without knowing the age of the individuals, by considering the actual age at metamorphosis (t_0), the proportion of a year available for growth (G) and the constant minimum number of growth years:

MALES	$(1.09 + t_0)/G$
FEMALES	$(1.55 + t_0)/G$

The minimum size at which *Bufo bufo* matures evidently is not constant, but depends on the population. Whether the minimum size at maturition is determined by genotype, by climatological factors, by other environmental conditions or by a combination of those is not sure, but the fact that the minimum size even varies between populations from the same area (cf. NET and GER) gives reason to suppose that the climate is not the only factor. As a consequence of variations among cohorts and among individuals, it is to be expected that not all individuals of the average minimum age indeed will achieve the average minimum size of maturation. Most likely, maturation within a given population is more dependent on the average minimum size

than on the average minimum age This is already mentioned by several authors (e.g. Hede 1976, Hede and Jørgensen 1978, Jørgensen and Billeter 1982) and is supported by the age structure of the adults, showing that the youngest adult age class is not the most numerous one. Furthermore, the fact that the estimated average maximal percentage of sexually mature toads in each age class corresponds broadly, though not entirely, to the age structure found, provides more evidence for this.

Summarizing, the results justify the conclusion that maturation of *Bufo bufo* may occur after having experienced a constant minimum number of growth years, but not before a given (population dependent) minimum size is reached

The estimated percentage of sexually mature toads in each age class did not correspond entirely to the age structure found in the samples. In most cases the presence of toads from the younger age classes was less than estimated, and toads from GER and SWI and females from NOR were even estimated to be able to mature at an earlier age than actually found. This is not surprising if one considers that the estimated percentages reflect an average situation, whereas the age structure found in the samples represents the situation in only one particular year. This is clearly demonstrated by the toads from GER, which may indeed mature at an earlier age than was found in this study (see chapter 2 and 5). The same was found for the males from SWI in 1984 (Hemelaar et al., in press). Furthermore, the estimated percentage represents a maximal percentage, the actual percentage of sexually mature toads of that age therefore being less in general. In addition, the lower catchability of smaller toads may keep the presence of the younger age classes in the sample down.

Growth

The growth rate of male and female toads at metamorphosis is approximately equal, but at each comparable adult age the body size and the phalangeal diameter of females is larger than that of males. The results showed that this sexual dimorphism becomes significant from the age of the youngest adult males or one year before Apparently, the later maturation of the females enables them to continue rapid growth for a longer period.

Among the populations the growth rates also differ. Although the annual increase of the phalangeal diameter in toads from populations with a short growing season is less than in those with a long growing season, the former toads actually grow faster per growth year and may reach larger

maximal dimensions than the latter. As the length of the growing season most likely is related to the environmental temperature, it actually means that toads from cold environments grow faster and reach larger dimensions than those from warm environments. Berven (1982*a*) found the same for *Rana sylvatica* and showed that indeed temperature was a factor determining growth rate and body size. However, the maximal dimensions and the growth rates of toads from NET and GER also differ, while living in a comparable temperature environment. This indicates that, whether temperature is a factor or not, other (additional) factors determine growth rate and maximal dimension in *Bufo bufo*. These factors might be genetical, as Berven (1982*a*) showed, although other environmental factors (for instance food quality and availability) cannot be excluded

According to Hede and Jørgensen (1978), growth of *Bufo bufo* decreases sharply after maturation, the final size attained by adult toads therefore being related to the size at which the animals become sexually mature This indicates that after maturation ingested food energy will be used mainly for maintenance and for reproduction in the next spring, at least in animals with an annual sexual cycle. Apparently, the growing season is too short, or the amount of ingested food energy generally is too small to be used for growth in addition.

However, the results of the present study show some growth in the younger adult age classes. This can be explained by the fact that not all members of a particular cohort attain adult sizes at the same time in the growing season According to Jørgensen (1975), initiation of the ovarian cycle in female *Bufo bufo* from Denmark does not occur later than July. In young females that reach adult sizes after July, the ovarian cycle is probably not initiated but postponed till the next year (Jørgensen et al. 1979). Then, young females which do not reach adult sizes in time to initiate the ovarian cycle may continue growing, thereby reaching larger sizes when maturing and migrating to the spawning site for the first time than those which did indeed attain adult sizes in time. If such is the case, the average size of females of a particular cohort would increase until all are sexually mature. The same could also apply to young males reaching adult sizes at a moment when their spermatogenesis is out of phase with the annual cycle of spermatogenesis in sexually mature males.

The fact that toads are full-grown (having reached 95% of their maximal size) at the age at which all toads are estimated to be sexually mature, supports this view. However, this does not hold for the females from NOR and, in particular, for those from SWI These females are full-grown at a

much older age. Observations on the population from SWI in 1983 and 1984 (Hemelaar et al., in press) show that the sexual cycle of most females is biennial, whereas the males breed every year. The same was found by Daugherty and Sheldon (1982) for *Ascaphus truei* from an altitude of 1500 metres. According to Jørgensen et al. (1979), the ovaries of female *Bufo bufo* enter a period of quiescence after spawning, during which the energy reserves of the organism are assumed to be restored before initiating the next ovarian cycle. As spawning at SWI occurs only in July, and hibernation already starts in September, the time apparently is (just?) too short to restore the energy reserves and to complete the vitellogenetic growth of the oocytes before hibernation. Females in a poor nutritional condition after spawning can, therefore, not participate in the next reproduction period of the population. During the season in which they do not reproduce, they may have left energy for growth, resulting in a continued increase of the average size of females of a cohort, even when all females of that cohort were estimated to be sexually mature. The same might apply to the females from NOR, although to a lesser extent. More females from this population are expected to be able to complete their sexual cycle within one season, because the growing season at NOR is longer than at SWI.

The continued growth in females from SWI and (to a lesser extent) NOR, as a result of a (partial) biennial sexual cycle, explains the larger variations in the reduction of the growth rate at maturation, the actual growth coefficient k and the number of growth years lapsed before being full-grown, as was found in females. In all probability, the values found for these parameters in females from NET and GER are representative for females with an annual sexual cycle, those found in females from SWI for females with a biennial cycle. As expected, females from NOR are intermediate with respect to these values. Therefore, to obtain more accurate information, the values of the parameters mentioned above should preferably not be averaged in female toads and caution should be taken when using a particular value in a population, without knowing the length of the female sexual cycle.

The fact that even the oldest females from SWI (12 years old) did not reach 95% of the maximal size (estimated to be reached after 15 years), may be due to the fact that their survival is too low to attain their potential maximum age and size. On the other hand, it is quite possible that the population structure under the extreme circumstances at high altitude is unstable. Indeed, toads from SWI may attain older ages than found in 1982.

In 1984 female toads were found up to 14 years old, and even an 18 years old female was found in 1983 (Hemelaar et al., in press).

Longevity

Toads from high altitude (SWI) and northern latitude (NOR) were found to reach older ages than those from low altitude and (more) southern latitudes (NET, GER, FRA), which agrees with Kuhnelt (1970) who stated that poikilothermic animals live longer in cold areas than in warm areas. The longer life-span of toads from SWI and NOR may be a result of two facts:

a. their ecological life time is longer, due to lower mortality as a result of, for instance, less predation, better food conditions or better hibernation conditions

b. their physiological life time is longer. Toads from SWI and NOR hibernate considerably longer than those from NET, GER and FRA, thus, live longer at minimum metabolic rates each year Furthermore, their metabolic rates during the growing season will be lower, as a consequence of the assumed lower environmental temperatures. It is quite possible that living at lower metabolic rates prolonges the physiological life time of a poikilothermic organism. Such a relationship between metabolic rates and longevity has been suggested before by Turner (1962).

The oldest toads from SWI and NOR, yet being older, have experienced less growth years than those from the other populations. Males from NOR and SWI should survive until they are 14 and 18, females until they are 16 and 21 years old, respectively, to have lapsed an equal number of growth years as those from NET and GER Although males and females from SWI may grow older than found in this study (13 and 18 years old respectively, Hemelaar et al., in press), it is not likely that they, nor the toads from NOR, may live that long.

Toads from FRA

Toads from FRA occupied an exceptional position in many respects. Their egg deposition occurred in two waves, which, according to local herpetologists, is common for this population. However, this is rather unusual for an explosive breeder such as *Bufo bufo.* Furthermore, body sizes are extremely large and more variable in comparison with those of toads from the other populations But most peculiar are the different

growth coefficient k and the different number of growth years lapsed at maturation

A different k may be found when the proportion of the year available for growth is wrongly estimated It can be calculated that toads from FRA should grow for only 3 7 months a year at the most to achieve an actual k similar to that of toads from the other populations. One could argue that this is possible when toads from FRA estivate during the warmest summer months, which is not considered in the estimations of the constants. However, the habitat of these toads is woody and situated at an altitude of 500 metres, which probably prevents extremely high temperatures and extreme aridity during summer, thereby making estivation less likely. Anyway, if they indeed do estivate for about 3.3 months, the growth coefficient k and the minimum number of growth years lapsed at maturation would reach values comparable to those of toads from the other populations.

On the other hand, there is a possibility that it was not *Bufo bufo bufo* which was studied in the South of France. Although Livet and Bons (1981) only reported the presence of *Bufo bufo bufo* in these surroundings, Parent (1976) mentioned the occurrence of *Bufo bufo spinosus* in the adjacent regions. The latter may grow much larger than *Bufo bufo bufo* and has thorns on the dorsal warts (Fretey 1975). *Bufo bufo spinosus* is very closely related to *Bufo bufo bufo.* Whether they are genetically different is still questionable Mertens and Wermuth (1960) considered *Bufo bufo spinosus* as a subspecies, de Lange (1973) as just a geographical race. Unfortunately, it cannot be decided whether the toads from FRA concerned *Bufo bufo spinosus, Bufo bufo bufo* or perhaps both, as no attention was paid to thorny warts during the field work However, if the toads from FRA indeed concerned *Bufo bufo spinosus,* the different growth coefficient k and the different minimum number of growth years experienced at maturation, support the idea of genetic differences between *Bufo bufo bufo* and *Bufo bufo spinosus.* In view of this, a skeletochronological study on distinct *Bufo bufo spinosus* might bring out more data, not in the least about its taxonomic status.

This thesis presents the results of a demographic study on *Bufo bufo* from different climatological conditions. Most of the results are obtained by skeletochronological analysis of the phalanges of the toads.

The first three chapters of this thesis describe a method to determine individually the age of the animals. In CHAPTER 1 hematoxylin-stained cross-sections of decalcified phalanges of toads from a temperate oceanic climate were analysed for structures which could be useful for age determination. Using different phalanges of the same individuals, captured and recaptured in several years, it was demonstrated that in the phalanges of those adults year rings are formed, reflecting the annual rhythm of growth of the toad. The additional structures that were found in some cross-sections did not cause problems in discerning the year rings.

Since in long bones like phalanges initially formed year rings may have been resorbed by remodelling of the bone, accurate age determination is only possible when, additionally to the number of year rings present, the number of year rings resorbed is also known For this purpose, CHAPTER 2 presents a method to determine the number of year rings resorbed in phalanges of adult *Bufo bufo* from a temperate oceanic climate. The method is based on both the pattern of year rings in a particular phalanx of the adults and the diameter of this phalanx in first-year toads towards hibernation. The degree of resorption of year rings appeared to be low in the male toads studied. In most males the first year ring, or remnants of it, were present.

Because there was reason to suppose that this method is less applicable to female toads and to toads from populations in which the degree of resorption of year rings is higher, the method was improved. The improved method and its application to populations from different latitudes and altitudes is described in detail in CHAPTER 3 It was shown that the period of metamorphosis is reflected in the phalanges as an hematoxylinophilic line, the so-called line of metamorphosis. The pattern of year rings in phalanges of toads in which the line of metamorphosis was present -and, thus, no year ring had been resorbed- formed the basis for estimating the number of year rings resorbed in the phalanges of the remaining toads. In 82-100% of the toads from the five populations studied this number could be estimated, and, consequently, their ages could be determined. The number

of year rings resorbed varied from 0 to 3, the percentage of toads in which at least one year ring had been resorbed from 4% to 76%. There were strong indications that the interpopulational differences and the sexual dimorphism found with respect to resorption are due to differences in the age at which maturity is reached.

The last three chapters deal with the demography of *Bufo bufo* from different climates. In CHAPTER 4 attention was paid to paired toads in a spawning population from a temperate oceanic climate. It was demonstrated that female toads mature at an older age than male toads, giving one reason why males outnumber females in the spawning population. Females may reach an older age too. No indications were found that older and/or larger male would have a better chance to reproduce (see also chapter 5). Mate selection based on age or size could not be established either Body size generally increases with age in this spawning population, although considerable overlap was found between the size distributions in the adjacent age classes. At each comparable adult age female toads are larger than male toads

From a subsequent study on the same spawning population in the following year, additional information about this population was obtained, which is presented in CHAPTER 5. The skeletochronological data showed that the sexual dimorphism concerning size develops before both sexes attain maturity. The age of maturation varied among individuals of the same sex As a consequence, not all members of the younger age classes did migrate to the spawning site during the reproduction period. The maximal proportion of sexually mature toads in each age class was estimated. Furthermore, the growth history of toads was given for each cohort separately. Among the cohorts, differences in growth at comparable ages were found, which were thought to be caused by variations in environmental conditions.

In CHAPTER 6 several demographic aspects of toads from different climatological conditions are presented, such as activity patterns, age structure and size distribution of the spawning population, annual growth, age and size at maturation and maximal proportion of sexually mature toads in each age class. The sexual dimorphism concerning size, annual growth, longevity, age and size at maturation, reported in the preceding two chapters, were confirmed for each population. Interpopulation differences were also found with respect to these and several other parameters. Toads from populations living at high altitude or northern latitude mature later and at larger sizes, may live longer, grow faster at equal sizes, and may

reach larger sizes than those living in lowland or southern latitude. It was demonstrated that at high altitude and northern latitude the older age at maturation is merely a result from the shorter growing season; the larger sizes at maturation, the faster growth and the longer life-span most likely are due only partially to the lower temperatures. When the actual time spent on growth each year was considered, growth was found to proceed equally among most populations. Therefore, the sexual dimorphic growth coefficient found was assumed to be a constant which describes all sexual dimorphic linear growth in common toads. The time to be spent on growth before the minimum size of maturation is reached, calculated with the growth equations, also appeared to be constant among most populations. With this constant it is possible to estimate the average minimum age at maturation in any population of common toads, without knowing the age of the animals.

DEMOGRAFISCH ONDERZOEK AAN BUFO BUFO L. (ANURA, AMPHIBIA) UIT VERSCHILLENDE KLIMATEN MET BEHULP VAN SKELETTOCHRONOLOGIE

Het hier beschreven onderzoek presenteert een demografische studie aan de Gewone Pad (*Bufo bufo*), afkomstig uit gebieden in Europa met verschillende klimaten. De resultaten werden voornamelijk verkregen door analyse van dwarsdoorsneden van phalangen (teenkoten) van padden. Het eerste deel van het proefschrift beschrijft een methode om de leeftijd van padden individueel te bepalen. In het tweede deel worden de hiermee verzamelde gegevens van padden afkomstig uit verschillende klimaten gepresenteerd.

Voor demografische studies aan amfibieën is een methode voor individuele leeftijdsbepaling van groot belang. Kennis van de leeftijd van individuen van een populatie biedt de mogelijkheid om inzicht te krijgen in populatieparameters, zoals de maximale levensduur, de leeftijd waarop de dieren geslachtsrijp worden, de mortaliteit in de verschillende leeftijdsklassen, de groei op verschillende leeftijden enzovoorts. Een vroeger veel gebruikte methode was de indeling van dieren in leeftijdsgroepen aan de hand van hun lichaamslengte. De nauwkeurigheid van deze methode laat echter te wensen over, omdat de lengteverdelingen van opeenvolgende leeftijdsgroepen elkaar meestal aanzienlijk overlappen, ten gevolge van de grote variatie in lichaamslengte tussen individuen van dezelfde leeftijd.

In het skelet van amfibieën zijn gelaagde structuren aanwezig die de periodieke groei van het dier weerspiegelen. Bestudering van deze periodieke groei in het skelet van een dier wordt ook wel skelettochronologie genoemd. De gelaagde strukturen zijn zowel in platte beenderen als in pijpbeenderen aanwezig en kunnen op verschillende manieren zichtbaar worden gemaakt. Erg duidelijk zijn ze in het periostaal beenweefsel van ontkalkte en met haematoxyline gekleurde dwarscoupes uit de diaphyse van phalangen. Omdat door het wegnemen van een phalanx een dier niet gedood hoeft te worden, biedt dit de mogelijkheid om skelettochronologie te betrekken in populatiedynamische studies aan amfibieën.

In het periostaal bot van phalangen bestaan de gelaagde strukturen uit relatief brede concentrische ringen beenweefsel, de zogenaamde groeiringen, die van elkaar worden gescheiden door relatief smalle lijnen, de zogenaamde rustlijnen. Gebleken is dat onder andere in phalangen van *Bufo bufo* uit

een continentaal klimaat slechts één groeiring en een rustlijn per jaar worden gevormd. De groeiring wordt gevormd gedurende het groeiseizoen en de rustlijn weerspiegelt de overwintering van het dier. Groeiring en rustlijn samen worden aangeduid als een jaarring.

De leeftijd van een dier hoeft echter niet gelijk te zijn aan het aantal jaarringen dat in het periostaal bot van een phalanx aanwezig is Er kan namelijk herstrukturering van beenweefsel optreden, waarbij periostaal bot kan worden vervangen door endostaal bot. De herstrukturering begint vanuit de mergholte, hetgeen betekent dat de vroegst gevormde jaarringen kunnen resorberen. Voor de bepaling van de leeftijd van een individu is het dan ook nodig om tevens het aantal geresorbeerde jaarringen te kennen.

In HOOFDSTUK 1 werden phalangen van adulte padden uit een gematigd zeeklimaat onderzocht op de aanwezigheid van jaarringen. Aan de hand van phalangen die in verschillende jaren werden weggenomen van dezelfde individuen, kon worden aangetoond dat in deze adulten inderdaad jaarringen worden gevormd De jaaringen konden over het algemeen duidelijk worden onderscheiden van andere gelaagde strukturen die in het periostaal bot van de phalanx aanwezig waren

In HOOFDSTUK 2 is een methode beschreven om het aantal geresorbeerde jaarringen vast te stellen in adulte mannetjes uit een gematigd zeeklimaat. Daartoe werd gebruik gemaakt van het patroon van jaarringen in een bepaalde phalanx van deze adulten, en van de diameter van deze phalanx in eerstejaars padden vlak voor hun overwintering In meer dan 90% van de onderzochte dieren kon zo de mate van resorptie van jaarringen worden vastgesteld. De mate van resorptie van jaarringen in de betreffende phalanx van deze mannetjes bleek gering te zijn. In de meeste dieren was de eerste jaarring aanwezig of tenminste restanten ervan.

Er waren aanwijzingen te veronderstellen dat deze methode minder succesvol zou zijn bij toepassing op populaties waarin de mate van resorptie groter is. De methode werd daarom verbeterd. De verbeterde methode en de toepassing ervan op populaties uit verschillende klimaten zijn beschreven in HOOFDSTUK 3. De resultaten toonden aan dat de periode van metamorfose als een herkenbare struktuur wordt vastgelegd in het periostaal bot van phalangen van het dier. Deze struktuur wordt de metamorfoselijn genoemd. Op basis van het patroon van jaarringen in phalangen van padden waarin deze metamorfoselijn aanwezig was -en dus geen jaarring kon zijn geresorbeerd- werd het aantal geresorbeerde jaarringen in de overige padden bepaald. In 82%-100% van de padden uit de onderzochte populaties was dit mogelijk. Het aantal geresorbeerde jaarringen varieerde

van 0 tot 3, het percentage padden waarin tenminste één jaarring volledig was geresorbeerd van 4% tot 76%. Tussen de beide geslachten en tussen de bestudeerde populaties werden verschillen gevonden in de mate van resorptie van jaarringen. Naar alle waarschijnlijkheid zijn deze veroorzaakt door verschillen in de leeftijd waarop de geslachtsrijpheid is bereikt.

Skelettochronologische analyse van dwarsdoorsneden van phalangen blijkt een nauwkeurige methode te zijn om de leeftijd van *Bufo bufo* te bepalen. Omdat de diameter van iedere jaarring in feite de diameter van de phalanx op een bepaalde leeftijd weergeeft, biedt skelettochronologie tevens een unieke mogelijkheid om informatie te krijgen over de groei van ieder individu. In de laatste drie hoofdstukken wordt ingegaan op demografische aspecten van padden uit verschillende klimatologische omstandigheden, welke met name door skelettochronologie zijn verkregen.

HOOFDSTUK 4 presenteert de leeftijdsstruktuur van padden in amplexus, afkomstig van een populatie uit een gematigd zeeklimaat. Vrouwtjes blijken gemiddeld later geslachtsrijp te worden dan mannetjes. Ze kunnen ook een hogere leeftijd bereiken. De latere maturatie van de vrouwtjes verklaart grotendeels het feit dat het aantal vrouwtjes in de voortplantingspopulatie ruimschoots wordt overtroffen door het aantal mannetjes. Er werden geen aanwijzingen gevonden dat oudere en/of grotere mannetjes een grotere kans zouden hebben om zich voort te planten. Ook kon geen partnerkeuze worden vastgesteld die op leeftijd of lichaamslengte zou zijn gebaseerd. Verder werd aangetoond dat de lichaamslengte van padden uit deze populatie toeneemt met de leeftijd, hoewel de lengteverdelingen in de opeenvolgende leeftijdsklassen elkaar aanzienlijk overlapten. Op iedere vergelijkbare adulte leeftijd zijn de vrouwtjes groter dan de mannetjes.

Meer informatie over dezelfde populatie werd verkregen door een voortzetting van het onderzoek in het daaropvolgende jaar. De resultaten hiervan zijn in HOOFDSTUK 5 beschreven. Aangetoond werd dat het sexueel dimorfisme wat grootte betreft zich ongeveer een jaar voordat de eerste mannetjes geslachtsrijp worden begint te ontwikkelen. Voorts kon worden geconcludeerd dat niet alle dieren op dezelfde leeftijd geslachtsrijp worden. Aangezien alleen geslachtsrijpe dieren tijdens de voortplantingsperiode naar de voortplantingsplaats trekken, betekent dit dat niet alle dieren van de jongere leeftijdsklassen in de voortplantingspopulatie aanwezig zullen zijn. Het maximale percentage geslachtsrijpe padden in iedere leeftijdsklasse werd geschat. Verder werden er tussen de cohorten (= groep dieren uit hetzelfde geboortejaar) verschillen in groei geconstateerd, welke vermoedelijk zijn

ontstaan als gevolg van jaarlijks variërende uitwendige omstandigheden

HOOFDSTUK 6 presenteert van *Bufo bufo* uit verschillende klimaten een aantal populatiekarakteristieken, zoals aktiviteitspatronen, leeftijdsstruktuur en lengteverdeling van de voortplantingspopulatie, jaarlijkse groei, minimumleeftijd en -grootte bij maturatie en maximaal percentage van geslachtsrijpe dieren in iedere leeftijdsklasse. Het sexueel dimorfisme, dat in de populatie uit een gematigd zeeklimaat (hoofdstuk 4 en 5) werd gevonden met betrekking tot diverse parameters, werd in iedere populatie aangetoond. Bovendien bleken er duidelijke verschillen tussen de populaties te bestaan. Padden die in noordelijke streken en/of op grote hoogte leven worden later geslachtsrijp en zijn dan groter, ze kunnen ouder worden, groeien sneller en kunnen groter worden dan padden die in meer zuidelijke streken en/of in laagland leven. De latere maturatie van eerst genoemde padden blijkt een gevolg te zijn van de kortere groeiseizoenen in deze streken De verschillen in de andere parameters zijn waarschijnlijk slechts gedeeltelijk te wijten aan verschillen in omgevingstemperaturen tussen de gebieden Analyse van de skelettochronologische gegevens per geslacht toonde dat de groei van padden uit de verschillende gebieden gelijk verloopt, wanneer alleen de werkelijke tijd die per jaar aan groei kan worden besteed in aanmerking wordt genomen. Dit leidde tot de conclusie dat de gevonden groeicoëfficiënt, die voor beide geslachten verschillend is, een sexueel dimorfe constante is, waarmee vermoedelijk het verloop van alle sexueel dimorfe lineaire groeiprocessen in de Gewone Pad kan worden beschreven Met behulp van de formules voor de groei van de padden werd voor beide geslachten en in iedere populatie de tijd berekend die aan groei moet worden besteed, voordat de minimum grootte van maturatie bereikt is Deze tijd blijkt een (sexueel dimorfe) constante te zijn, waarmee het mogelijk is om de gemiddelde minimumleeftijd van maturatie te schatten in iedere populatie van de Gewone Pad, zonder de leeftijd van de dieren te kennen

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Agnes Hemelaar, geboren op 26 juli 1951 te Rucphen (N.B.).

Het diploma Gymnasium β heb ik in 1969 aan het St. Gertrudislyceum te Roosendaal behaald. Aansluitend volgde ik de studie Biologie aan de Katholieke Universiteit te Nijmegen. Het kandidaats examen B4 (biologie en scheikunde) heb ik in november 1972 afgelegd, het doctoraal examen cum laude in 1977. Hoofdvak was Dieroecologie, bijvakken waren Organische Chemie en Aquatische Oecologie.

Van september 1977 tot september 1978 was ik in het kader van de T.A.P.-regeling verbonden aan de afdeling Dieroecologie van de Katholieke Universiteit te Nijmegen. In januari 1979 volgde een tijdelijke aanstelling aan voornoemde afdeling als wetenschappelijk medewerkster. Naast wetenschappelijk onderzoek heb ik een belangrijk deel van de tijd besteed aan onderwijstaken. De arbeidsovereenkomst werd na 5 verlengingen op 1 januari 1984 beëindigd.

In 1985 heb ik me laten omscholen tot systeemanalist, in welke functie ik sedert 1 maart 1986 werkzaam ben bij Philips N.V., Nijmegen.

STELLINGEN

1

De conclusies van Davies en Halliday ten aanzien van de relatieve lichaamslengte van mannetjes en vrouwtjes van *Bufo bufo* (L) in amplexus tijdens de afzetting en het percentage gefertiliseerde eieren zijn gebaseerd op onterechte toepassingen van de verklarende statistiek

Davies NB, Halliday TR (1977) Nature 269 56-58

2

Een adequate beoordeling van de toxicologische effecten van zware metalen op zoetwatervissen wordt bemoeilijkt door een toenemende verzuring van het oppervlaktewater

McDonald DG (1983) Can J Zool 61 691-703

3

De conclusie van Lee en Vince dat het sulfoxide zuurstofatoom van sparsomycine niet bijdraagt aan de ribosomale binding, omdat het S-deoxo-analoog een aan sparsomycine vergelijkbare bindingsaffiniteit zou hebben, wordt niet ondersteund door de resultaten van Liskamp et al en Ottenheijm et al

Lee CK, Vince R (1978) J Med Chem 21 176-179

Liskamp RMJ, Colstee JH, Ottenheijm HCJ, Lelieveld P, Akkerman W (1984) J Med Chem 27 301-306

Ottenheijm HCJ, Broek LAGM van den, Ballesta JPG, Zylicz Z In Progress in Medicinal Chemistry (23) Ellis GP, West GB (Eds) Elsevier, Amsterdam (in press)

4

De door van den Tweel en Schalk ontwikkelde methode ter observatie van de architectuur en groeidynamiek van wortelstelsels zou tot gevolg moeten hebben dat wortelsystemen meer aandacht krijgen in plantenoecologisch onderzoek

Tweel PA van den, Schalk B (1981) Plant and Soil 59 163-165

5

Het verloop van de handeling in Richard Wagner s Gesamtkunstwerk Der Ring des Nibelungen wordt in hoge mate bepaald door de getoonde vaardigheid in het vangen van padden (Das Rheingold, derde scene)

6

Dat de Muurhagedis *Podarcis muralis* (Laurenti) nog steeds niet definitief uit ons land is verdwenen is niet te danken aan de daadkracht en zorg van de verantwoordelijke overheidsinstanties

Bonnemayer J, Dietvorst P (1979) Rapport 160 en 167, Afdeling Dieroecologie, KUN

Hanekamp G, Stumpel AHP (1983) Intern rapport, Werkgroep Amfibieen en Reptielen Nederland, RIN, Arnhem

7

Bij bestudering van effecten van milieuverzuring op het uitkomen van amfibieeneieren wordt onvoldoende rekening gehouden met mogelijke veranderingen in de structuur van het vitelline membraan en de omhullende geleilagen

Dunson WA, Connell J (1982) J Herpetol 16 314-316

Clark KL, LaZerte BD (1985) Can J Fish Aquat Sci 42 1544-1551

8

Resultaten van recent telemetrisch onderzoek aan overwinterende *Bufo bufo* (L.) rechtvaardigen de introductie van de term "actieve hibernatie".

Gelder JJ, Olders JHJ, Bosch JWG, Starmans PW. Holarctic Ecology (in press).

9

Pas gepromoveerden met post-promotionele depressies dienen door deskundigen begeleid te worden, in het bijzonder betreffende de besteding van hun herwonnen vrije tijd.

10

Goed werk vraagt tijd.

Dit proefschrift.

Agnes Hemelaar, Nijmegen, 26 juni 1986.